UNIVERSITA' DI MESSINA - UNIVERSIDAD AUTONOMA DE MADRID

Facultad de Ciencias

Departamento de Química Orgánica

Carbohydrate Functionalized Oligo(phenylene ethynylenes) (OPEs): Synthesis, Photophysical and Biological Properties.

Tesis Doctoral

Elisa Deni

Supervisors:

Prof. Anna Barattucci (Università degli Studi di Messina)

Prof. Mª Carmen Carreño García (Universidad Autonoma de Madrid)

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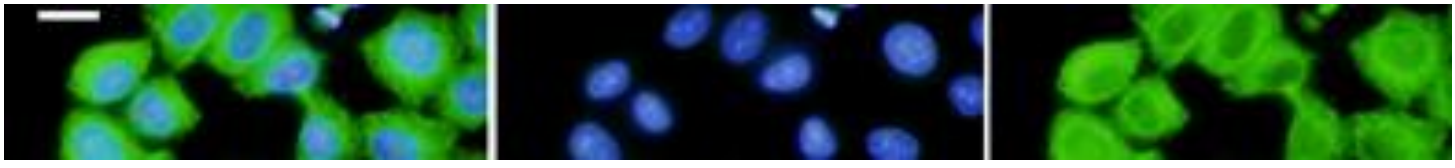

Ci vuole un gran fisico per correre dietro ai sogni. Sono velocissimi, scappano, si nascondono, si mimetizzano e a volte sembrano sparire del tutto. Ma in fondo cos'è un sogno?

Un sogno è un momento di silenzio, ma anche un urlo fortissimo, un sogno è un'occasione, un treno che percorre un binario che in certi momenti sembra non finire mai, un binario dove a volte le fermate sembrano durare ore, un binario che a volte si interrompe per prendere strade secondarie, un binario percorso da un treno dove in certi momenti diventa tutto buio, e dove la paura è la tua unica compagna.

La passione è il motore dei nostri sogni.

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RIASSUNTO DI TESI IN COTUTELA

Disciplina: Chimica

Indirizzo: Chimica Organica

Candidata: DENI, Elisa

Titolo: ***« Carbohydrate Functionalized Oligo(phenylene ethynylenes) (OPEs): Synthesis, Photophysical and Biological Properties. »***

Realizzata nei:

Departamento de Química Orgánica Módulo 01. Facultad de Ciencias, Universidad Autónoma de Madrid, España.

Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Università di Messina, Italia

Relatrici della Tesi: Prof. M. Carmen Carreño García (UAM)
Dr. Anna Barattucci (Univ. MESSINA)

« Carbohydrate Functionalized Oligo(phenylene ethynylenes) (OPEs): Synthesis, Photophysical and Biological Properties.»

Questa tesi di dottorato è stata realizzata in cotutela tra l'Università di Messina, Italia, presso il Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali e diretta dalla Dr. Anna Barattucci, e l'Universidad Autónoma de Madrid, Spagna, presso il Departamento de Química Orgánica, diretta dalla Prof. M. Carmen Carreño.

INTRODUZIONE

I derivati degli Oligo(fenilenetinileni) (OPEs; sigla in inglese), costituiscono un gruppo di coloranti luminescenti con una struttura coniugata molto stabile, con la forma caratteristica di bastoncino.[1] Questi composti posseggono proprietà di enorme interesse applicativo in numerosi ambiti, fondamentalmente dovute al fatto che il loro scheletro possiede una estesa coniugazione. Le loro proprietà possono essere modulate con l'inserimento di differenti sostituenti agli anelli aromatici dello scheletro coniugato con conseguente modifica del sistema elettronico del sistema.[2] La suddetta facilità nel variare le loro proprietà elettroniche ha determinato l'applicazione di questi derivati in vari campi, dalla sensoristica[3] all'elettronica, [4] ma anche in campo biologico.[5]

L'uso di sonde fluorescenti, d'altra parte, ha ormai facilitato enormemente l'interpretazione di alcuni processi biologici a livello molecolare e cellulare.[6] Perchè vi siano applicazioni biomediche, ai composti che possono essere utilizzati come sonde fluorescenti si richiedono caratteristiche strutturali ben definite, visto che devono subire eccitazione per irraggiamento ad una determinata lunghezza d'onda che sia compatibile con i substrati biologici, così come avere un'emissione in un determinato intervallo di λ. In più, perchè siano applicabili in campo biologico, queste molecole devono essere bio- e fotostabili, oltre a possedere la capacità di mantenere la propria farmacocinetica senza alterazioni nel tempo. Questa ultima caratteristica viene spesso ritrovata in strutture coniugate che posseggono frammenti di amminoacidi e carboidrati.

Visti questi presupposti, si è pensato che l'introduzione di carboidrati semplici nella struttura degli OPEs avrebbe potuto aggiungere alle loro note caratteristiche strutturali un frammento naturale che ne avrebbe facilitato l'utilizzo come sonde fluorescenti in processi biologici. L'ntroduzione di residui zuccherini avrebbe potuto inoltre aumentare la solubilità in acqua di questi composti, oltre ad incrementarne la biocompatibilità. D'altra parte, la proporzione relativa tra frammenti aromatici (idrofobici) e carboidrati

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[1] Bunz, U. H. F. Chem. Rev. 2000, 100, 1605−1644.

[2] (a) Yamaguchi, Y.; Shimoi, Y.; Ochi, T.; Wakamiya, T.; Matsubara, Y.; Yoshida, Z.-I. J. Phys. Chem. A 2008, 112, 5074−5084. (b) Zhi, Y.-G.; Lai, S.-W.; Chan, Q. K.-W.; Law, Y.-C.; Tong, G.S.-M.; Che, C.-M. Eur. J. Org. Chem. 2006, 3125−3139. (c) Yamaguchi, Y.; Tanaka, T.; Kobayashi, S.; Wakamiya, T.; Matsubara, Y.; Yoshida, Z.-i. J. Am. Chem. Soc. 2005, 127, 9332−9333.

[3] (a) Kim, U.-I.; Suk, J.-m.; Naidu, V. R.; Jeong, K.-S. Chem. Eur. J. 2008, 14, 11406−11414. (b) Thomas, S. W., III; Joly, G. D.; Swager, T. M. Chem. Rev. 2007, 107, 1339−1386. (c) Kim, I.-K.; Erdogan, B.; Wilson, J. N.; Bunz, U. H. F. Chem. Eur. J. 2004, 10, 6247−6254.

[4] (a) Kaliginedi, V.; Moreno-García, P.; Valkenier, H.; Hong, W.; García Suarez, V. M.; Buiter, P.; Otten, J. L. H.; Hummelen, J. C.; Lambert, C. J.; Wandlowski, T. J. Am. Chem. Soc. 2012, 134, 5262−5275. (b) Yamaguchi, Y.; Kobayashi, S.; Wakamiya, T.; Matsubara, Y.; Yoshida, Z.-i. Angew. Chem., Int. Ed. Engl. 2005, 44, 7040−7044.

[5] (a) Hill, E. H.; Evans, D. G.; Whitten, D. G. Langmuir 2013, 29, 9712−9720. (b) Dascier, D.; Ji, E.; Parthasarathy, A.; Schanze, K. S.; Whitten, D. G. Langmuir 2012, 28, 11286−11290. (c) McRae, R. L.; Phillips, R. L.; Kim, I.-B.; Bunz, U. H. F.; Fahrni, C. J. J. Am. Chem. Soc. 2008, 130, 7851−7853.

[6] (a) He, X.; Wang, K.; Cheng, Z. Nanomed. Nanobiotechnol. 2010, 2, 349−366. (b) Kobayashi, H.; Ogawa, M.; Alford, R.; Choyke, P. L.; Urano, Y. Chem. Rev. 2010, 110, 2620−2640. (c) Rao, J.; Dragulescu-Andrasi, A.; Yao, H. Curr. Opin. Biotechnol. 2007, 18, 17−25.

(idrofilici) negli OPEs glicosilati, che è possibile modulare attraverso l'uso di un'adeguata metodologia sintetica, avrebbe potuto essere essenziale per successive applicazioni biologiche. Come conseguenza di queste premesse sono stati pianificati, per questa tesi di dottorato, gli obiettivi qui di seguito elencati.

OBIETTIVI

SINTESI

- Il primo obiettivo di questa tesi di dottorato è stato incentrato sulla **Síntesi di derivati di Oligo (fenilen etinileni), OPEs,** di differente grandezza molecolare (fino a tre unità fenilenetinileniche), e contenenti due frammenti glicosidici derivati dal glucosio, oltre che sostituenti differenti (OMe, NMe_2, $^{+}NMe_3$) agli anelli aromatici. Si è inoltre pianificata la sintesi di altri derivati analoghi con unità di BODIPY e azobenzene. Alcune delle strutture sintetizzate sono incluse in Figura 1.

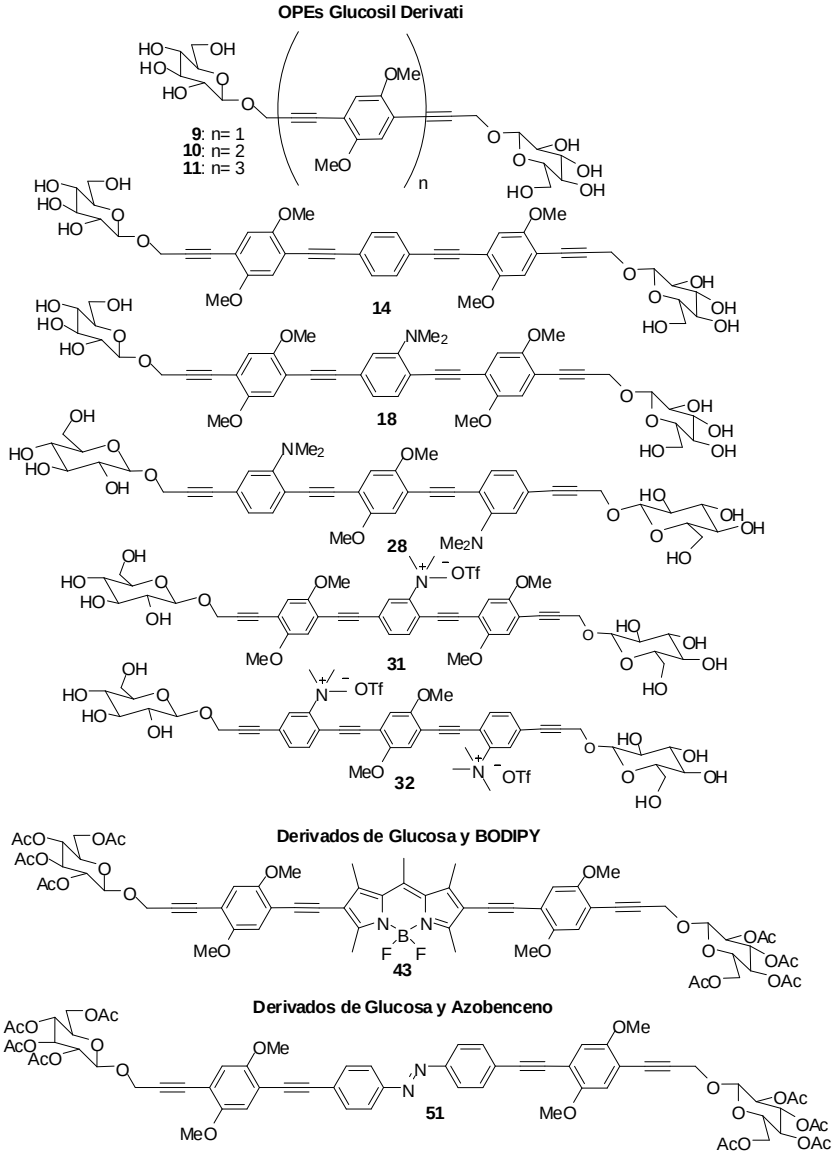


Figura 1.- OPEs Glucosil Derivati sintetizzati

E’ stata inoltre realizzata la sintesi di analoghi galattosidici, col fine ultimo di studiare l’influenza della natura dello zucchero sulle proprietà degli OPEs risultanti (Figura 2). Alla stessa maniera, sono stati sintetizzati OPEs contenenti due residui di Isobornil solfossido.

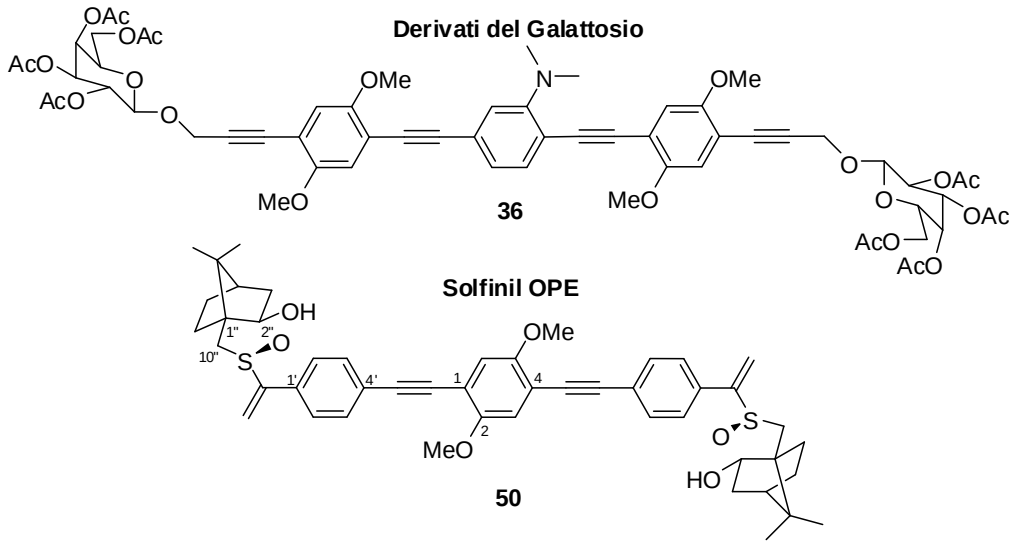


Figura 2.- Galattoside e Solfossido derivati degli OPEs sintetizzati

STUDIO DI PROPRIETA’

- Studio di **propietà fotofísiche** dei composti ottenuti e valutazione, in alcuni casi, della loro capacità di generare Ossigeno singoletto.

- Studio delle **propietà biologiche**. Inizialmente questo studio è stato incentrato sulla valutazione della capacità di questi composti di attraversare la membrana cellulare del tessuto epidermoide del carcinoma della laringe (HEp-2), con il fine ultimo di determinare la loro possibile applicazione come sonde biologiche fluorescenti. Questa parte del lavoro è stata effettuata in collaborazione col gruppo di ricerca della Prof. Maria Teresa Sciortino dell’Università di Messina. Il distinto rapporto esistente negli OPEs sintetizzati tra resti idrofilici (carboidrati) e resti idrofobici (areni sostituiti) permetterà di riconoscere la loro relativa influenza sulla bioaffinità.

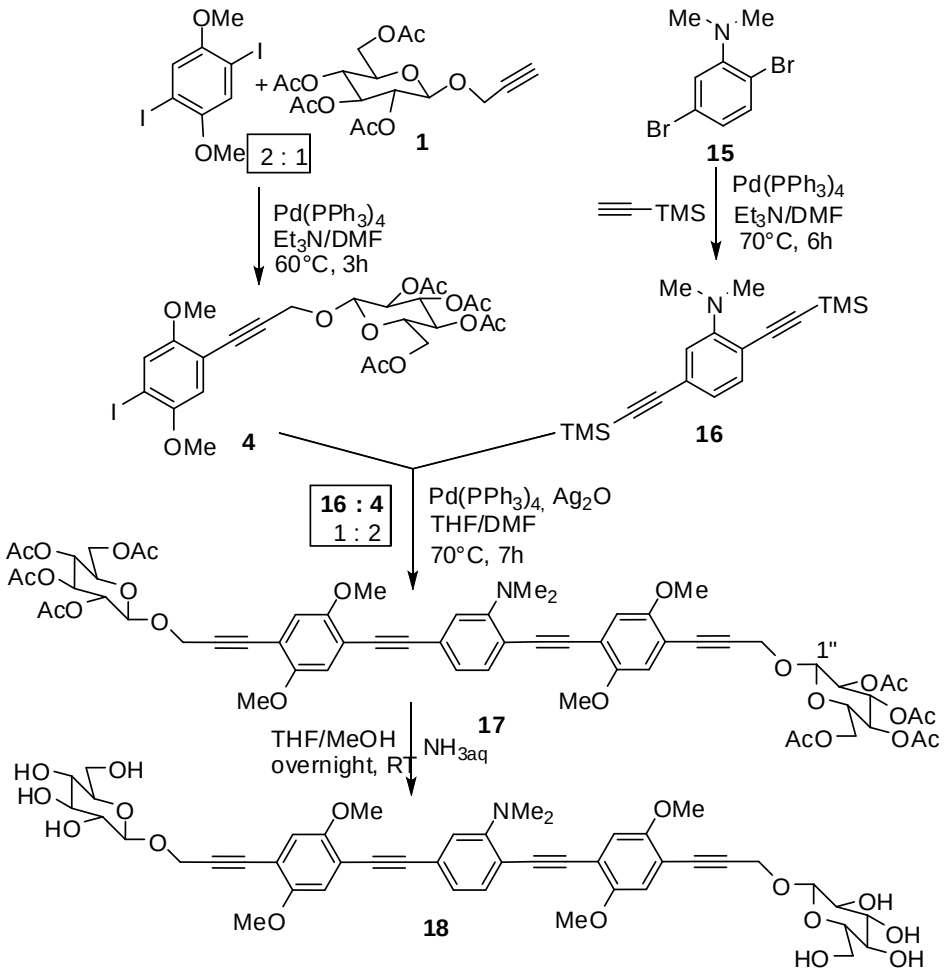
- Studio della capacità di alcuni degli OPEs ottenuti di agire come **fotosensibilizzatori** per un’applicazione in Terapia Fotodinamica (PDT). Con questo fine, sono state utilizzate varie linee cellulari: Cellule di cheratinociti umani (HaCaT), cellule di cancro della cervice umana (HeLa) e cellule del tessuto epidermoide del carcinoma della laringe (HEp-2). Questa parte di lavoro è stata portata avanti in collaborazione con il gruppo di ricerca della Prof. Ángeles Juarranz, del Departamento de Biología dell’ Universidad Autónoma de Madrid

Sintesi di OPEs funzionalizzati con carboidrati.

La metodologia applicata per la costruzione degli scheletri carboniosi dei suddetti oligo fenilenetinileni (OPEs) è stata basata sull’uso iterativo di una reazione di tipo Sonogashira catalizzata da Pd(0), in assenza di rame. Si è così riusciti ad occoppiare differenti frammenti con resti acetilenici con anelli

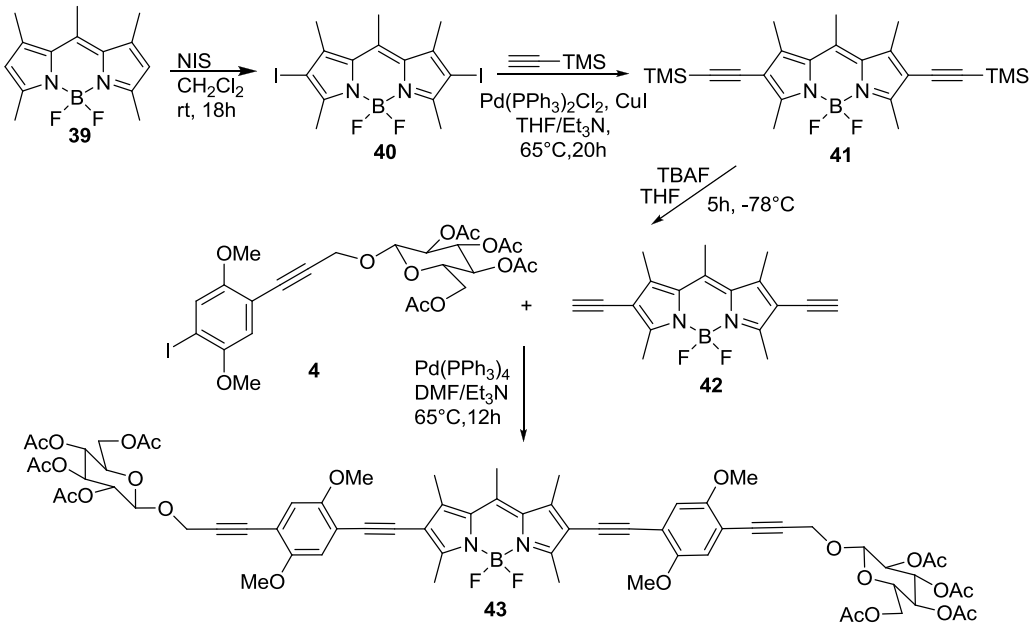
aromatico iodo e bromo sostituiti che, inoltre, contenessero i diversi gruppi funzionali presenti nelle strutture finali. La unità di acetilene iniziale reca il glicoside corrispondente.

Come esempio rappresentativo, si riporta la sintesi dell'OPE **18** nello schema 1. A partire dall'1,5-dimetossi-1,4-diiodo benzene è stato introdotto sull'anello aromatico, in maniera controllata, un equivalente di glucoside propargilico peracetilato **1**. Il prodotto **4** derivato da questo monoaccoppiamento possiede ancora un resto di iodoarene che può essere sottoposto ad una nuova funzionalizzazione. Per disegnare una sintesi il più possibile convergente, è stata effettuata la doppia funzionalizzazione della 2,5-dibromo dimetil anilina **15**, con un eccesso di trimetilsililacetilene, in presenza di $Pd(PPh_3)_4$ e NEt_3 in DMF, ottenendo il derivato **16**. L'unione dei due frammenti **4** (2 equivalenti) e **16**, è stata effettuata senza la necessità di eliminare i gruppi TMS da quest'ultimo, utilizzando nella reazione di Sonogashira Ag_2O, oltre che il cataizzatore di Pd (0). Il ruolo dell'ossido di argento è fondamentale in quanto facilita la desililazione simultaneamente all'accoppiamento. Una volta ottenuto l'OPE adeguadamente funzionalizzato, è stata necessaria solo la deprotezione dei gruppi acetato del glucoside, ottenuta con resa quantitativa per trattamento del prodotto **17** con una soluzione di idrossido ammonico, utilizzando una miscela di THF e Metanolo per solubilizzare il substrato peracetilato. La metodologia si è dimostrata molto generale ed è stata applicata alla sintesi di una larga varietà di OPEs, solo sintetizzando previamente gli acetinil areni adeguadamente sostituiti. A partire dal propargil galattoside analogo a **1**, si è potuta ottenere la sintesi della serie corrispondente mediante una metodologia simile.



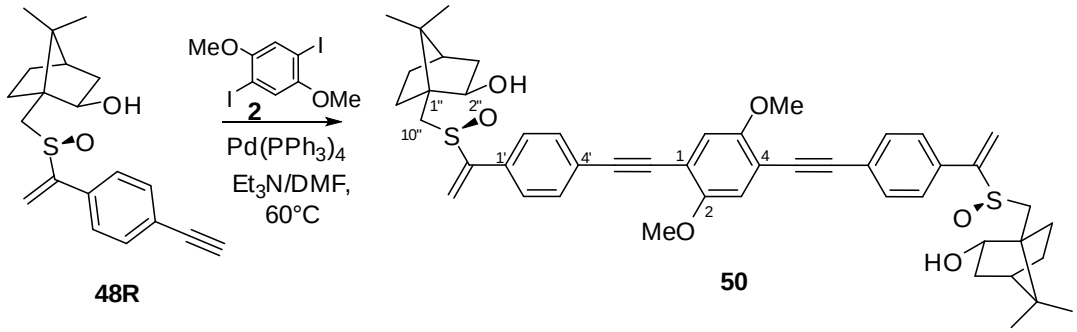
(*Schema 1*)

La ricerca sviluppata ha anche permesso di accedere al substrato **43** indicato nello Schema 2, che contiene un frammento centrale di BODIPY, sintetizzato a partire dal 2,4-dimetilpirrolo, applicando una metodologia sintetica già descritta. L'introduzione di due residui acetilenici nel BOPIPY ha permesso di accedere al substrato **42**, il cui accoppiamento con lo iodoarene glicosilato **4**, ha portato alla molecola obiettivo **43**. (*Schema 2*)



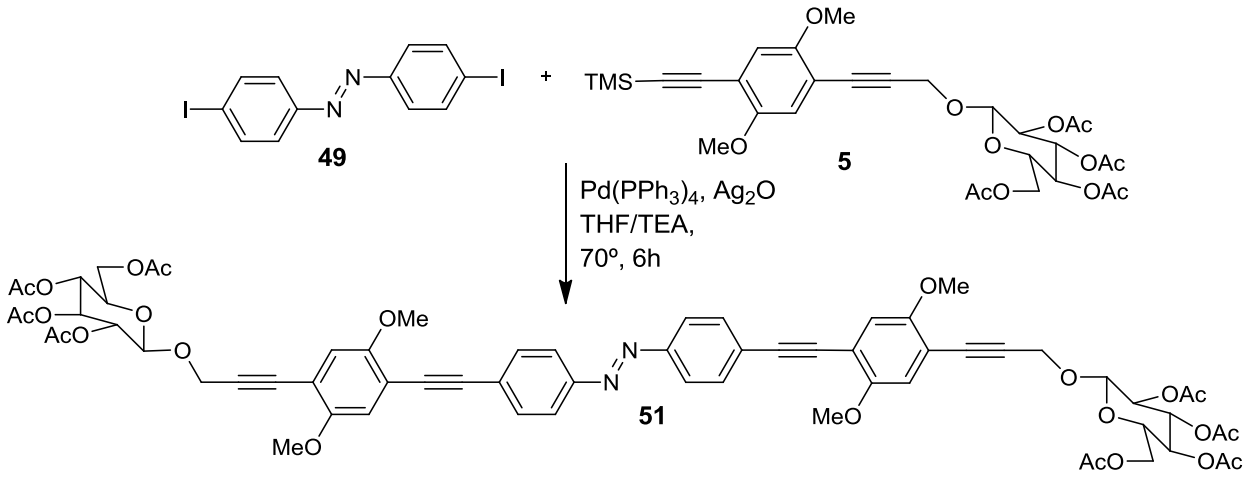
Schema 2

Il metodo ha portato anche alla sintesi del solfossido **50** (Schema 3), e al suo diastereomero (epimero ai due atomi di zolfo), grazie al doppio accoppiamento tra acetinilsolfinil derivato **48R** e il 2,5-dimetossi-1,4-iodobenzene **2**.



Schema 3

Nel caso caso dell'azoderivato **51**, la sintesi è stata compiuta in maniera analoga, come indicato nello Schema 4, mediante il doppio accoppiamento tra il diiodoazobenzene **49** e il bisacetilene **5**, che recava l'unità di carboidrato.



Schema 4

STUDIO DI PROPRIETA'

La seconda parte di questa tesi di dottorato si è incentrata sullo studio di proprietà fotofisiche dei composti sintetizzati.

PROPRIETA' FOTOFISICHE

Sono stati registrati gli spettri di assorbimento (UV/vis) degli OPES sintetizzati in soluzione acquosa tamponata (fosfato) così come dei precursori acetilati in diclorometano: è stata in ogni caso osservata un'intensa banda di assorbimento nella regione 340-390 nm, dovuta alla transizione permessa□□-□* dei sistemi aromatici coniugati. In tutti i casi si è osservato un comportamento spettroscopico le cui differenze vengono qui di seguito riassunte. L'aumento della coniugazione causa uno spostamento batocromico della banda menzionata, com'era atteso. D'altra parte, anche il coefficiente di estinzione molare aumenta con la coniugazione. L'eccitazione di questi composti, nell'intervallo 280-420 nm, produce una emissione nella regione del blu e blu-verde. La presenza del gruppo NMe_2, fortemente elettrondonatore, all'anello centrale dell'oligomero **17** sposta la luminescenza verso il rosso e diminuisce leggermente la resa quantica, che comunque si mantiene elevata (0,57). Il profilo d'emissione di questo composto suggerisce l'esistenza di uno stato eccitato con un trasferimento di carica parziale attribuibile alla transizione $n \rightarrow \pi^*$.

PROPRIETA' BIOLOGICHE

L'internalizzazione degli OPEs sintetizzati in cellule di tipo HEp-2 (tessuto epidermoide del carcinoma della laringe) è stata studiata con l'utilizzo della microscopia a fluorescenza. Gli esperimenti effettuati hanno messo in evidenza un comportamento di questi composti direttamente relazionato alle caratteristiche strutturali e spettroscopiche appena commentate. Nonostante l'oligomero **18**, che possiede un anello centrale con un sostituente di tipo dimetilamminico e i due residui di glucosio con i gruppi OH liberi, sia risultato essere il composto più biocompatibile e promettente per essere utilizzato come sonda fluorescente nelle cellule, l'elevata emissione e le alte rese quantiche osservate per tutti gli OPEs studiati, così come la

loro alta stabilità fanno di questi glucosidi adeguati candidati per l'utilizzo come coloranti in microscopia a fluorescenza.

Un altro studio di interesse effettuato in questa tesi corrisponde alla valutazione della capacità del substrato **17** e dell'analogo con due anelli contenenti ognuno un gruppo NMe_2, di agire come fotosensibilizzatore in Terapia Fotodinamica (PDT). La PDT è un procedimento minimamente invasivo che può produrre l'eradicazione di malattie neoplastiche combinando l'effetto di un fotosensibilizzatore, di irraggiamento con luce di una determinata lunghezza d'onda e di ossigeno. Lo studio biologico dei nuovi ammino oligo(fenilen-etinileni) sintetizzati in questo lavoro, e tra loro dell'OPE **18**, ha messo in evidenza la loro capacità di agire come fotosensibilizzatori in combinazione con la luce UVA. I saggi sono stati effettuati su due linee cellulari tumorali (HEp-2 y HeLa) provenienti dal carcinoma della laringe e della cervice, rispettivamente. Una volta irradiati, questi composti causano un blocco mitotico che conduce alla finale morte cellulare. In più, sono risultati efficaci a concentrazioni fino a 3 μm.

Conclusioni:

- Il lavoro realizzato per questa tesi di dottorato ha permesso di mettere a punto una metodologia sintetica iterativa e convergente che conduce alla costruzione efficiente dello scheletro degli OPEs obiettivo. La versatilità del processo ha condotto a glicosidi contenenti anelli aromatici centrali diversamente sostituiti, attraverso un accoppiamento di tipo Sonogashira catalizzato da Pd(0), in assenza di catalizzatore di Cu, tra acetileni adeguatamente funzionalizzati e bromo o iodo areni.

- Le proprietà fisiche, come l'alta resa quantica e la produzione di Ossigeno singoletto, sono state valutate, avendo potuto stabilire in origine la loro capacità di produrre luminescenza con emissione nella regione blu, blu-verde.
- La loro stabilità, biocompatibilità, efficace internalizzazione nelle cellule e la loro buona risposta, anche a basse concentrazioni, fanno dei derivati amminici sostituiti fotosensibilizzatori promettenti in Terapia fotodinamica.
- Gli ammino derivati sono capaci di produrre un efficace effetto foto dinamico quando irradiati con luce UVA provocando la morte cellulare, anche con bassa produzione di ossigeno singoletto.
- Lo studio effettuato in questo lavoro ha messo in evidenza, per la prima volta, la possibilità di utilizzare gli OPEs come fotosensibilizzatori e può essere un punto di partenza per estendere le applicazioni di questi composti luminescenti nel campo della Terapia Fotodinamica (PDT).

Publicaciones:

AUTORI: Anna Barattucci, Maria Chiara Aversa, Elisa Deni, Teresa Papalia, Paola Bonaccorsi
TÍTOLO: **Synthesis of Enantiomerically Pure Bis-Sulfinyl Substituted Phenylene Ethynylenes**
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DOI: 10.1002/hlca.201400144

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TÍTOLO: **Oligo(phenylene ethynylene) Glucosides: Modulation of Cellular Uptake Capacity Preserving Light ON**
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AUTORI*:* Elisa Deni, Alicia Zamarrón, Paola Bonaccorsi, M. Carmen Carreño, Ángeles Juarranz, Fausto Puntoriero, Maria Teresa Sciortino, María Ribagorda, Anna Barattucci
TÍTOLO: **Glucose-functionalized Amino-OPEs as Biocompatible Photosensitizers in PDT**
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Comunicazioni a Congressi

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POSTER: *XXXV Convegno Nazionale della Divisione di Chimica Organica*, Sassari, 9-13 Settembre
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RESUMEN DE TESIS EN COTUTELA

Disciplina: Química

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Candidata: DENI, Elisa

Título: ***a***

Realizada en:

Departamento de Química Orgánica Módulo 01. Facultad de Ciencias, Universidad Autónoma de Madrid, España.

Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Università di Messina, Italia

Directores de Tesis: Prof. M. Carmen Carreño García (UAM)
Dr. Anna Barattucci (Univ. MESSINA)

«* Carbohydrate Functionalized Oligo(phenylene ethynylenes) (OPEs): Synthesis, Photophysical and Biological Properties.*»

Esta tesis doctoral ha sido realizada en cotutela entre la Universidad de Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Università di Messina, Italia, dirigida por la Dra. Anna Barattucci, y en la Universidad Autónoma de Madrid, en el Departamento de Química Orgánica, dirigida por la Profesora M. Carmen Carreño.

INTRODUCCIÓN

Los derivados de Oligo(fenilenetinileno) (OPEs; siglas en inglés) constituyen un grupo de colorantes luminiscentes con una estructura conjugada muy estable, en forma de barra.[7] Estos compuestos poseen propiedades de enorme interés aplicado en distintos campos que se deben fundamentalmente a que su esqueleto presenta una conjugación extendida. Las propiedades se pueden modular a voluntad mediante la introducción de distintos sustituyentes en los anillos aromáticos del esqueleto conjugado que modifican las características electrónicas de los sistemas.[8] Esta facilidad para variar sus propiedades electrónicas ha determinado que se hayan encontrado numerosas aplicaciones para estos derivados entre las que cabe destacar su uso como sensores,[9] en electrónica,[10] y en el campo de las aplicaciones biológicas.[11]

Por otra parte, el uso de sondas fluorescentes ha facilitado enormemente la interpretación de algunos procesos biológicos a nivel molecular y celular.[12] Las aplicaciones biomédicas de los compuestos que pueden utilizarse como sondas fluorescentes requieren unas características estructurales concretas ya que las moléculas fluorescentes han de sufrir la excitación por irradiación a una determinada longitud que sea compatible con los sustratos biológicos, así como presentar una emisión en un determinado rango de λ. Para poder ser aplicadas en el campo biológico, estas moléculas han de ser bio y foto estables además de poseer la capacidad de mantener su farmacocinética sin alteraciones a lo largo del tiempo. Esta última característica se encuentra con mucha frecuencia en estructuras conjugadas que poseen fragmentos de amino ácidos o carbohidratos.

La introducción de carbohidratos sencillos en las estructuras de los OPEs, añadiría a las características estructurales de los mismos un fragmento natural que facilitaría su utilización como sondas fluorescentes en procesos biológicos. La introducción de restos de azúcar podría también aumentar la solubilidad en agua de estos compuestos así como incrementar su biocompatibilidad. Por otra parte, la proporción relativa entre fragmentos aromáticos (hidrofóbicos) y carbohidratos (hidrofílicos) en los OPEs glicosilados, que puede modularse mediante metodología sintética, podría ser esencial para futuras aplicaciones biológicas. De

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[7] Bunz, U. H. F. Chem. Rev. 2000, 100, 1605−1644.

[8] (a) Yamaguchi, Y.; Shimoi, Y.; Ochi, T.; Wakamiya, T.; Matsubara, Y.; Yoshida, Z.-I. J. Phys. Chem. A 2008, 112, 5074−5084. (b) Zhi, Y.-G.; Lai, S.-W.; Chan, Q. K.-W.; Law, Y.-C.; Tong, G.S.-M.; Che, C.-M. Eur. J. Org. Chem. 2006, 3125−3139. (c) Yamaguchi, Y.; Tanaka, T.; Kobayashi, S.; Wakamiya, T.; Matsubara, Y.; Yoshida, Z.-i. J. Am. Chem. Soc. 2005, 127, 9332−9333.

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[11] (a) Hill, E. H.; Evans, D. G.; Whitten, D. G. Langmuir 2013, 29, 9712−9720. (b) Dascier, D.; Ji, E.; Parthasarathy, A.; Schanze, K. S.; Whitten, D. G. Langmuir 2012, 28, 11286−11290. (c) McRae, R. L.; Phillips, R. L.; Kim, I.-B.; Bunz, U. H. F.; Fahrni, C. J. J. Am. Chem. Soc. 2008, 130, 7851−7853.

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acuerdo con estas premisas se plantearon, para esta tesis doctoral, los objetivos que se indican a continuación.

OBJETIVOS

SÍNTESIS

- El primer objetivo de esta tesis doctoral se centró en la **Síntesis de derivados de Oligo (fenilen etinilenos), OPEs,** de diferente magnitud molecular (hasta tres unidades de fenilenetinileno), conteniendo dos fragmentos glicosídicos derivados de glucosa además de distintos sustituyentes (OMe, NMe_2, $^{+}NMe_3$) en los anillos aromáticos. También se planteó la síntesis de otros derivados análogos con unidades de BODIPY y azobenceno. Algunas de las estructuras sintetizadas se incluyen en la Figura 1.

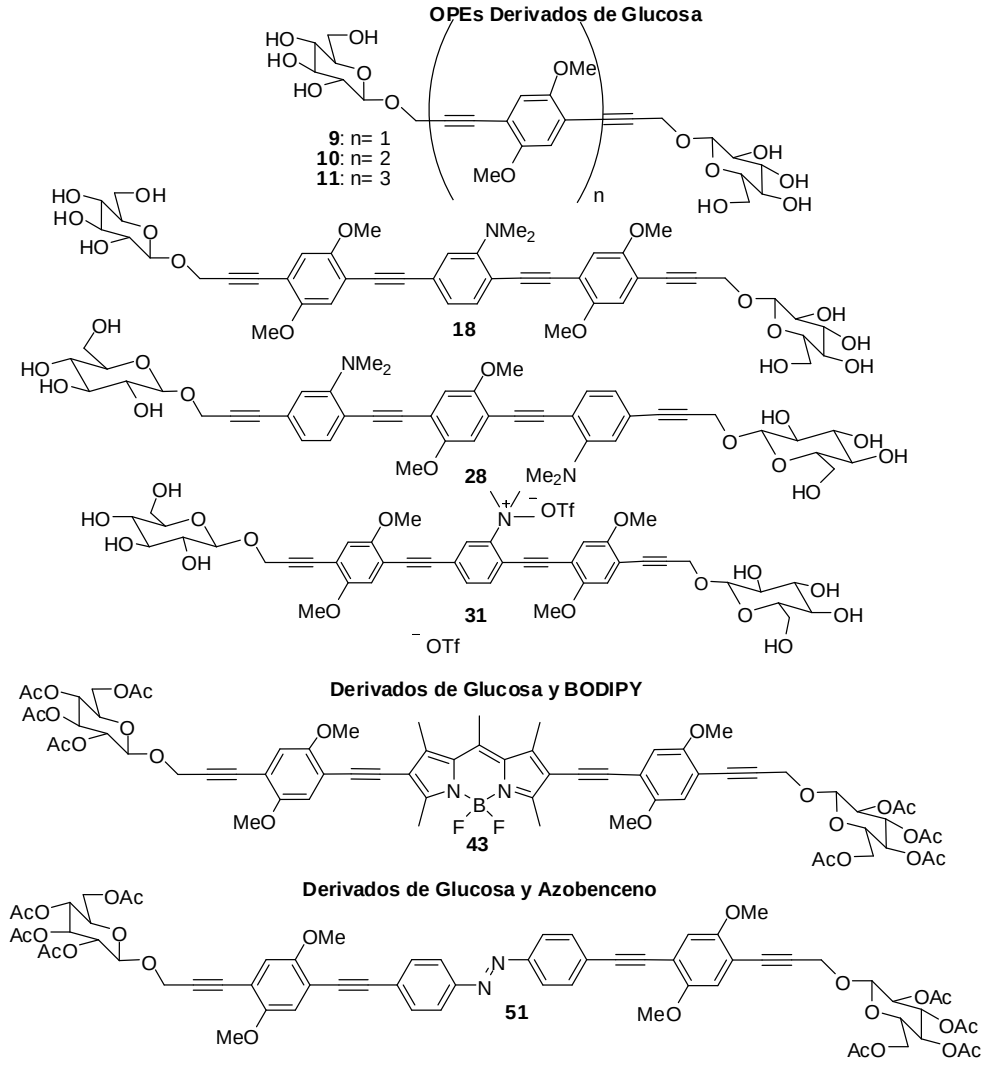


Figura 1.- Glucósidos derivados de OPEs sintetizados

La síntesis de galactósidos análogos también se llevó a cabo con el fin de estudiar la influencia de la naturaleza del azúcar en las propiedades de los OPEs resultantes (Figura 2). Asimismo, se sintetizaron OPEs conteniendo dos restos de isobornil sulfóxido.

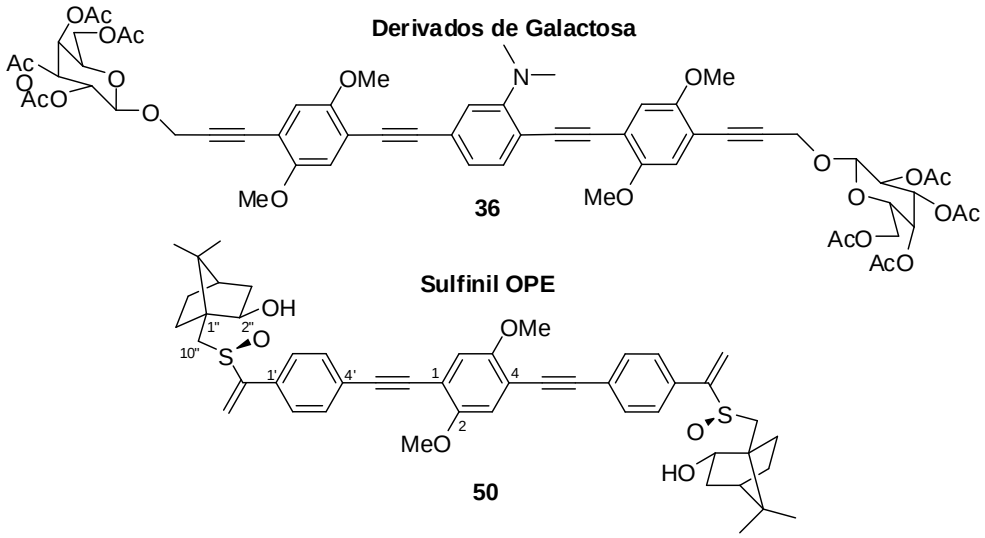


Figura 2.- Glalactósido y sulfóxido derivados de OPEs sintetizados

ESTUDIO DE PROPIEDADES

- Estudio de las **propiedades fotofísicas** de los compuestos obtenidos y evaluación, en algunos casos, de su capacidad para generar Oxígeno singlete.

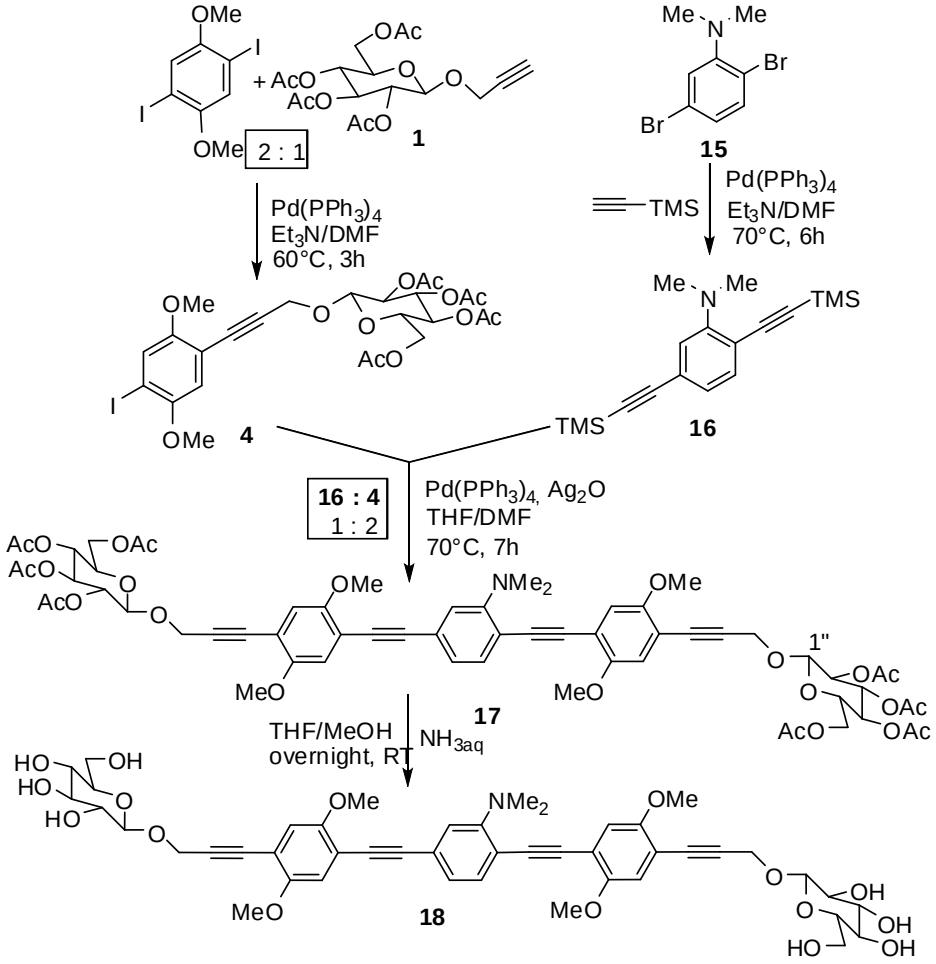
- Estudio de **propiedades biológicas**. Inicialmente este estudio se centró en evaluar la capacidad de estos compuestos para atravesar las membranas celulares del tejido epidermoide del carcinoma de laringe (HEp-2), con el fin de determinar su posible aplicación como sondas biológicas fluorescentes. Esta parte del trabajo se llevó a cabo en colaboración con el grupo de investigación de la Prof. Maria Teresa Sciortino, de la Universidad de Messina. La distinta relación existente en los OPEs sintetizados de restos hidrofóbicos (arenos sustituidos) e hidrofílicos (carbohidratos) permitirá conocer su influencia relativa sobre la bioafinidad.

- Estudio de la capacidad de algunos de los OPEs obtenidos de actuar como fotosensibilizadores para su aplicación en Terapia Fotodinámica (PDT). Con este fin se utilizaron distintas líneas celulares: Células de keratinocitos humanos (HaCaT), células humanas de cáncer cervical (HeLa) y células del tejido epidermoide del carcinoma de laringe (HEp-2). Esta parte del trabajo se llevó a cabo en colaboración con el grupo de investigación de la Prof. Ángeles Juarranz, del Departamento de Biología de la Universidad Autónoma de Madrid.

Síntesis de OPEs funcionalizados con carbohidratos.

La metodología aplicada para la construcción de los esqueletos carbonados de estos oligo fenilenetinilenos (OPEs) se basó en el uso iterativo de una reacción de tipo Sonogashira catalizada por Pd(0), en ausencia de cobre. Se lograron así acoplar distintos fragmentos con restos acetilénicos con anillos aromáticos Iodo o bromo sustituidos que, además, contenían los distintos grupos funcionales presentes en las estructuras finales. La unidad inicial de acetileno aportaba el glicósido correspondiente.

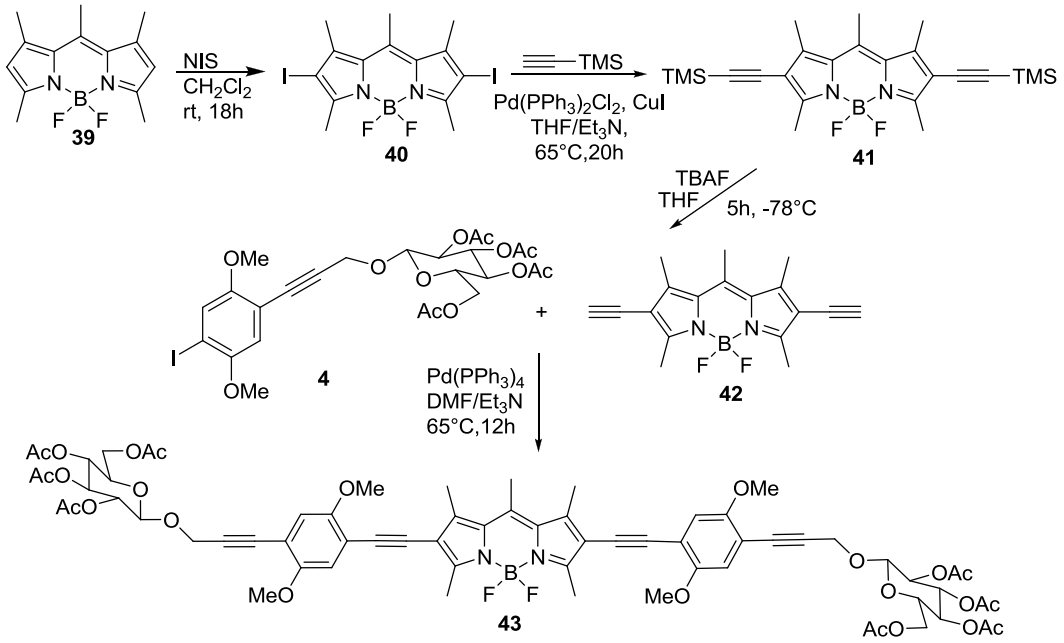
Como ejemplo representativo, se recoge la síntesis del OPE **18** en el Esquema 1. Así, a partir de 2,5-dimetoxi-1,4-diiodo benceno se introdujo un equivalente del glucósido propargílico peracetilado **1** de forma controlada en el anillo aromático. El producto **4** resultante de este monoacoplamiento, todavía contenía un resto de iodo areno susceptible de ser funcionalizado nuevamente. Para diseñar una síntesis lo más convergente posible, se llevó a cabo la doble funcionalización de la 2,5-dibromo dimetil anilina **15**, con exceso de trimetilsililacetileno, en presencia de $Pd(PPh_3)_4$ y NEt_3 en DMF, lográndose obtener el derivado **16**. La unión entre los fragmentos **4** (2 equivalentes) y **16**, se pudo llevar a cabo sin necesidad de eliminar los grupos TMS de este último, utilizando en la reacción de Sonogashira Ag_2O, además del catalizador de Pd (0). El papel de la plata es fundamental ya que debe facilitar la desililacón de forma simultánea al acoplamiento. Una vez obtenido el OPE adecuadamente funcionalizado, sólo se requería la desprotección de los acetatos del glucósido, lo que se logró con rendimiento cuantitativo por tratamiento del producto **17** con una disolución de hidróxido amónico utilizando una mezcla de THF y Metanol para solubilizar el sustrato peracetilado.



(*Esquema 1*)

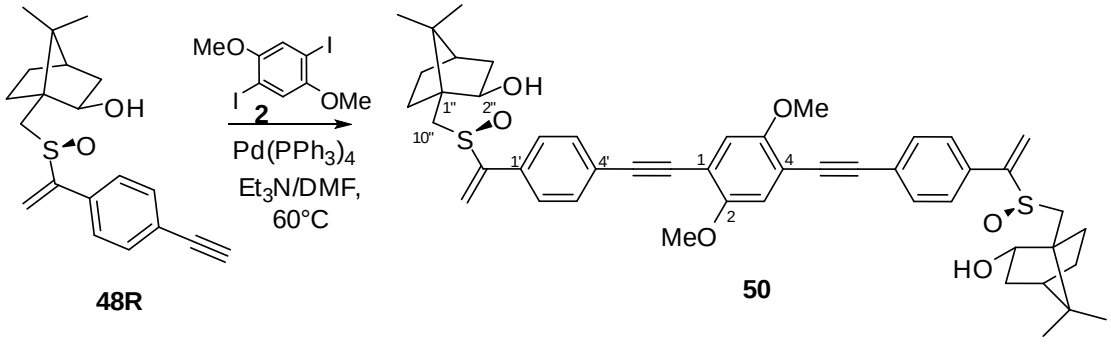
La metodología se mostró muy general y pudo se aplicada a la síntesis de una variedad de OPEs sin más que sintetizar previamente los acetinil arenos adecuadamente sustituidos. A partir del propargil galactósido análogo a **1**, se pudo llevar a cabo la síntesis de las series correspondientes mediante una metodología similar.

La investigación desarrollada permitió también acceder al sustrato **43** indicado en el Esquema 2, que contiene un fragmento central de BODIPY, sintetizado a partir de 2,4-dimetil pirrol, aplicando metodología descrita. La introducción de sendos acetilenos en el BOPIPY permitió acceder al sustrato **42**, cuyo acoplamiento con el iodo areno glicosilado **4**, condujo a la molécula objetivo **43**. (*Esquema 2*)



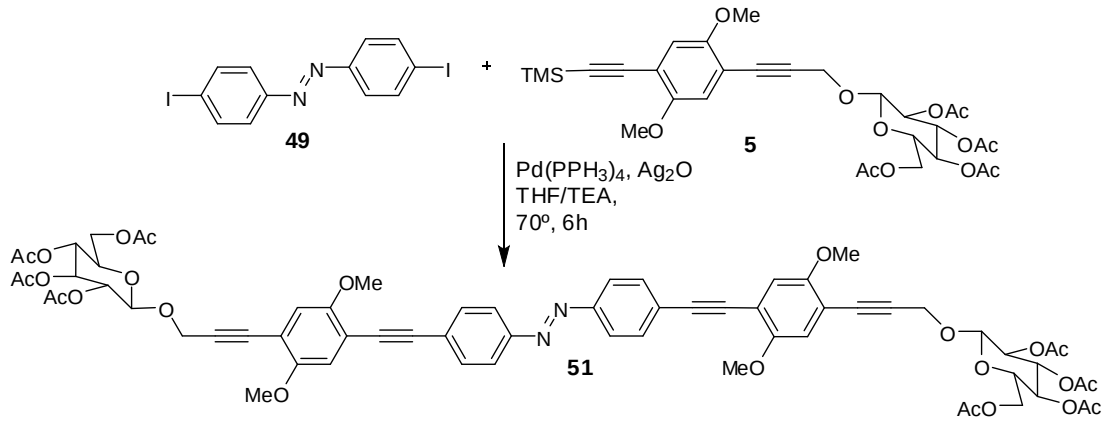
Esquema 2

El método condujo también a la síntesis del sulfóxido **50** (Esquema 3) y a su diastereomero (epimero a los dos azufre), gracias al doble acoplamiento entre el acetinil sulfinil derivado **48R** y el 2,5-dimetoxi-1,4-iodobenceno **2**.



Esquema 3

En el caso del azoderivado **51**, la síntesis se completó de forma análoga, según se indica en el Esquema 4 medianrte el doble acoplamiento entre el diiodoazobenceno **49** y el bisacetileno **5**, que aportaba la unidad de carbohidrato.



Esquema 4

ESTUDIO DE PROPIEDADES

La segunda parte de esta tesis doctoral se centró en el estudio de las propiedades fotofísicas de los compuestos sintetizados.

PROPIEDADES FOTOFÍSICAS

Se registraron los espectros de absorción (UV/vis) de los OPES sintetizados en disolución acuosa tamponada (fosfato) así como de los precursores acetilados en diclorometano, observándose una intensa banda de absorción en todos ellos en la región de 340-390 nm debida a la transición $\pi-\pi^*$ de los sitemas aromáticos conjugados, permitida. Se observó en todos los casos, un comportamiento espectroscópico cuyas diferencias se resumen a continuación. El aumento de la conjugación determina un desplazamiento batocrómico de la banda mencionada, como cabía esperar. Por otra parte, el coeficiente de extinción molar también aumenta con la conjugación. La excitación de estos compuestos en el rango de 280-420 nm, produce una emisión en la región azul y azul verde. La presencia del grupo NMe_2, fuertemente electrón donador, en el anillo central del oligómero **17** desplaza la luminiscencia hacia el rojo y disminuye ligeramente el rendimiento cuántico, que en cualquier caso continúa siendo elevado (0,57). El perfile de la emisión de este compuesto sugiere la existencia de un estado excitado con una transferencia de carga parcial que se pudo atribuir a la transición n $\rightarrow \pi^*$.

PROPIEDADES BIOLÓGICAS

La internalización de los OPEs sintetizados en células de tipo HEp-2 (tejido epidermoide del carcinoma de laringe) se ha estudiado por microscopía de fluorescencia. Los experimentos llevados a cabo han puesto de manifiesto un comportamiento directamente relacionado con las características estructurales y espectroscópicas antes comentadas para estos compuestos. Aunque el oligómero **18** que posee un anillo central con un sustituyente de tipo dimetilamino y los dos restos de glucosa con los grupos OH libres, ha resultado ser el compuesto más biocompatible y prometedor para ser utilizado como sonda fluorescente en las células, la elevada emisión y altos rendimientos cuánticos, observados en todos los OPEs estudiados, así como su estabilidad hacen de estos glucósidos candidatos adecuados para ser utilizados como colorantes en microscopía de fluorescencia.

Otro estudio de interés llevado a cabo en este trabajo corresponde a la evaluación de la capacidad de los sustratos **17** y el análogo con dos anillos que contienen sendos grupos NMe_2, de actuar como fotosensibilizadores en Terapia Fotodinámica (PDT). La PDT es un procedimiento mínimamente invasivo que puede producir la erradicación de enfermedades neoplásicas combinando el efecto de un fotosensibilizador, irradiación con una luz de longitud de onda determinada y oxígeno. El estudio biológico de los nuevos amino oligo(fenilen-etinilenos) sintetizados en este trabajo, entre ellos, el OPE **18**, ha puesto de manifiesto su capacidad para actuar como fotosensibilizadores en combinación con luz UVA. Los ensayos se realizaron sobre dos líneas tumorales celulares (HEp-2 y HeLa) derivadas del carcinoma de laringe y cervical, respectivamente. Una vez irradiados, estos compuestos disparan un bloqueo mitótico que conduce finalmente a la muerte celular. Además, resultaron efectivos en concentraciones de hasta 3 μm.

Conclusiones:

- El trabajo realizado en esta tesis doctoral ha permitido poner a punto una metodología sintética iterativa y convergente que conduce a la construcción de los esqueletos presentes en los OPEs objetivo. La versatilidad del proceso ha conducido a los glicósidos conteniendo distintos anillos aromáticos sustituidos centrales a través de acoplamientos de tipo Sonogashira catalizado por Pd(0), en ausencia de catalizador de Cu, entre acetilenos adecuadamente funcionalizados y bromo o iodo derivados aromáticos.

- Las propiedades fotofísicas tales como alto rendimiento cuántico y producción de Oxígeno singlete, han sido evaluadas habiéndose podido establecer su capacidad para producir luminiscencia con emisión en la región de azul y azul-verde.
- Su estabilidad, biocompatibilidad, eficaz inernalización en las células y su buena respuesta, incluso a baja concentración, hacen de los derivados amino sustituidos fotosensibilzadores prometedores aplicables en el campo de la Terapia fotodinámica.
- Estos amino derivados son capaces de producir un eficaz efecto fotodinámico al ser irradiados con luz UVA provocando la muerte celular incluso con baja producción de Oxígeno singlete.
- El estudio llevado a cabo en este trabajo ha puesto de manifiesto, por primera vez, la posibilidad de utilizar los OPEs como fotosensibilizadores y pude ser el punto de partida para extender las aplicaciones d eestos compuestos luminiscentes en el campo de la Terapia Fotodinámica (PDT).

Publicaciones:

AUTORES: Anna Barattucci, Maria Chiara Aversa, Elisa Deni, Teresa Papalia, Paola Bonaccorsi
TÍTULO: **Synthesis of Enantiomerically Pure Bis-Sulfinyl Substituted Phenylene Ethynylenes**
REVISTA: *Helvetica Chimica Acta*, **2014**, *97* (9), 1237–1243
DOI: 10.1002/hlca.201400144

AUTORES: Anna Barattucci, Elisa Deni, Paola Bonaccorsi, Maria Grazia Ceraolo, Teresa Papalia, Antonio Santoro,Maria Teresa Sciortino, and Fausto Puntoriero

TÍTULO: **Oligo(phenylene ethynylene) Glucosides: Modulation of Cellular Uptake Capacity Preserving Light ON**
REVISTA: *J. Org. Chem.*, **2014**, *79*, 5113–5120
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AUTORES: Elisa Deni, Alicia Zamarrón, Paola Bonaccorsi, M. Carmen Carreño, Ángeles Juarranz, Fausto Puntoriero, Maria Teresa Sciortino, María Ribagorda, Anna Barattucci
TÍTULO: **Glucose-functionalized Amino-OPEs as Biocompatible Photosensitizers in PDT**
REVISTA: *Eur. J. Med. Chem.*, **2016**, en prensa

Comunicaciones a Congresos

POSTER: *Simposio de Investigadores Jovenes*, Bilbao, 4-7 Noviembre 2014
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COMUNICACIÓN ORAL: ***Convegno Congiunto delle Sezioni Calabria e Sicilia***, Catania, 2-3 Diciembre 2013
O13: Sintesi di fili molecolari decorati con residui zuccherini e loro proprieta' foto fisiche.
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POSTER:*XXXV Convegno Nazionale della Divisione di Chimica Organica*, Sassari, 9-13 Septiembre
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List of Abbreviations

In this Ph. D. memory, the abbreviations used are listed in "Guidelines for Authors" (J. Org. Chem. 2013)

BODIPY: BoronDiPyrromethene

cat: Catalyst

DCE: 1,2-Dicloroethane

DMF: Dimethylformamide

DMSO: Dimethyl sulfoxide

equiv: Equivalente(s)

Et_3N: Triethylamine

HaCaT: Cultured Human Keratinocyte cells

HeLa: Human Cervical Carcinoma cells

HEp-2: Human epithelial type 2 cells

NMR: Nuclear Magnetic Resonance

OPEs: Oligo(Phenylene)ethynylenes

PPEs: Poly(Phenylene)ethynylenes

PS: Photosensitizer

PDT: Photodinamic Therapy

rt: room temperature

TBAF: tetrabuthyl ammonium fluoride

Tf: Trifluoromethansulfonate

THF: Tetrahydrofurane

TMS: Trimethylsylil

Tripan Blu: (3Z,3'Z)-3,3'-[(3,3'-dimethylbiphenyl-4,4'-diyl)di(1Z)hydrazin-2-yl-1-ylidene]bis(5-amino-4-oxo-3,4-dihydronaphthalene-2,7-disulfonic acid)

UV/Vis: Ultraviolet/Visible

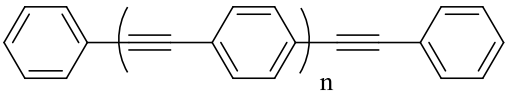
Chapters

Introduction and Objectives

Chapter 1

1.1.General features of Oligo(Phenylene)Ethynylenes (OPEs)

Oligo(phenylene-ethynylenes) (OPEs) and the corresponding polymers, Poly(phenylene-ethynylenes) (PPEs),represent a peculiar class of luminescent dyes with stable π-conjugated rigid rod-like skeletons.[1] In particular, their structure is a succession of aromatic rings linked by carbon-carbon triple bonds (figure 1). This feature guarantees a high electronic conjugation and, consequently, their photochemical properties evidence high quantum yields and characteristic luminescence. The synthetic approaches leading to the construction of these molecules allow easy functional group manipulation and make their functional properties tunable.



Oligo Phenylene Ethynylenes: OPEs

Figure 1

Thanks to the possibility of tuning their properties, OPEs and PPEs have attracted significant attention in the scientific community. In the last two decades, OPEs and PPEs have found a

[1]Bunz, U. H. F. *Chem. Rev.* **2000**, *100*, 1605–1644.

great variety of applications in several fields, from sensing[2] and electronics[3] to the biological one.[4]

1.2.Photophysical properties of OPEs

The photophysical properties of OPEs are directly connected to their extensive conjugation. Modulation of these properties depends on their structure and on substitution on the aromatic conjugated skeleton.

In 2005Yamaguchi,[5] reported a study about the photophysical properties of OPEs (quantum yield Φ_f, emission wavelength λ_{em}, lifetime τ, absorption wavelength λ_{abs}) and their behavior when the π-conjugated molecular rods consisting of *p*-(phenylene)ethynylene units were modified by donor (OMe) and/or acceptor (CN) groups situated at the aromatic rings. They reported three different cases: (1) side-donor modification systems (**SD** systems), (2) side-acceptor modification systems (**SA** systems), and (3) systems consisting of donor blocks and acceptor blocks (**BL** systems, figure 2).

[2] a) Kim, U.-I.; Suk, J.-m.; Naidu, V. R.; Jeong, K.-S. *Chem. Eur. J.* **2008**, *14*, 11406−11414; b) Thomas III, S. W.; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007,** *107*, 1339-1386; c) Kim, I.-K.; Erdogan, B.; Wilson, J. N.; Bunz, U. H. F. *Chem. Eur. J.* **2004**, *10*, 6247-6254.

[3]Kaliginedi, V.; Moreno-García, P.; Valkenier, H.; Hong, W.; García Suarez, V. M.; Buiter, P.; Otten, J. L. H.; Hummelen. J. C.; Lambert, C. J.; Wandlowski, T. *J. Am. Chem. Soc.* **2012**, *134*, 5262-5275.

[4]a) Mc Rae, R. L.; Phillips, R. L.; Kim, I.-B.; Bunz, U. H.; Fahrni, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 7851-7853; b) Disney, M. D. ; Zheng, J.; Swager, T.M., Seeberger*, P.H. *J. Am. Chem. Soc.* **2004**, *126*, 13343-13346.

[5]Yamaguchi, Y.; Tanaka, T.; Kobayashi, S.; Wakamiya, T.; Matsubara, Y.; Yoshida, Z.-i. *J. Am. Chem. Soc.* **2005**, *127*, 9332-9333.

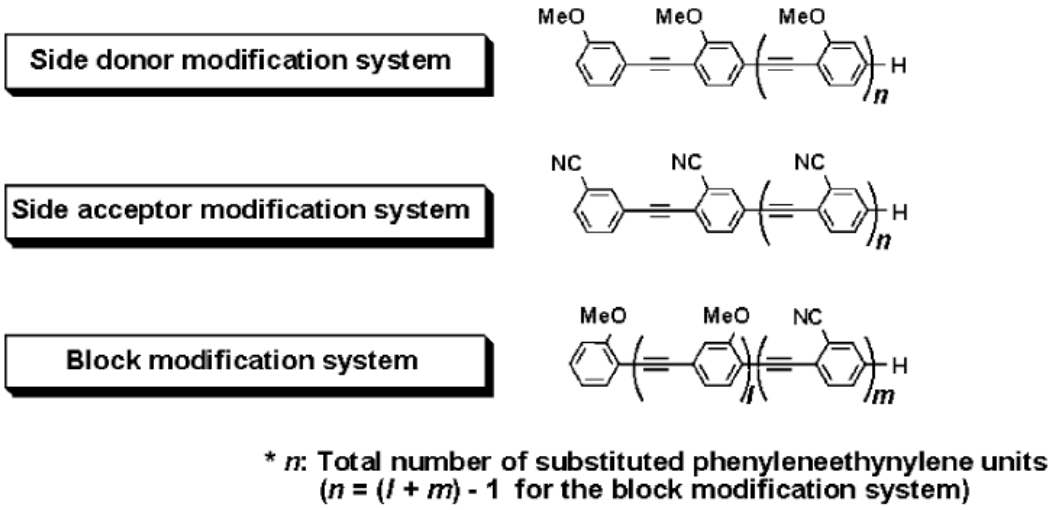


Figure 2

The best values in terms of Φ_f, were obtained for the block modification systems (**BL**,Φ_f>0.95). This result evidenced that the building a push-pull system by combination of electron donating and electron withdrawing groups in the same skeleton improved the photophysical properties of the OPEs. On the other side, the values of quntum yields obtained for the systems functionalized with –OMe in the side donor moiety (**SD**) or with –CN in the side acceptor fragment, (**SA**) were higher(Φ_f>0.90).The study of the trend of bathochromic shift of emission (λ_{em}) compared to the elongation of aromatic chain gave very interesting results since the largest **OPEs** systems showed λ_{em} shifted toward higher wavelengths.

Rodríguez *et al.* reported in 2003the new class of bended OPEs depicted in figure 3. These structures had an increased conjugation due to the link of new subunits with 1,3,5 substituted ethynyl benzenes shown a defined the conjugated geometry.[6]

[6]Gonzalo Rodrıguez,J.; Esquivias, J.; Lafuente, A.; Diaz, C.; *J. Org. Chem.* **2003**, *68*, 8120-8128.

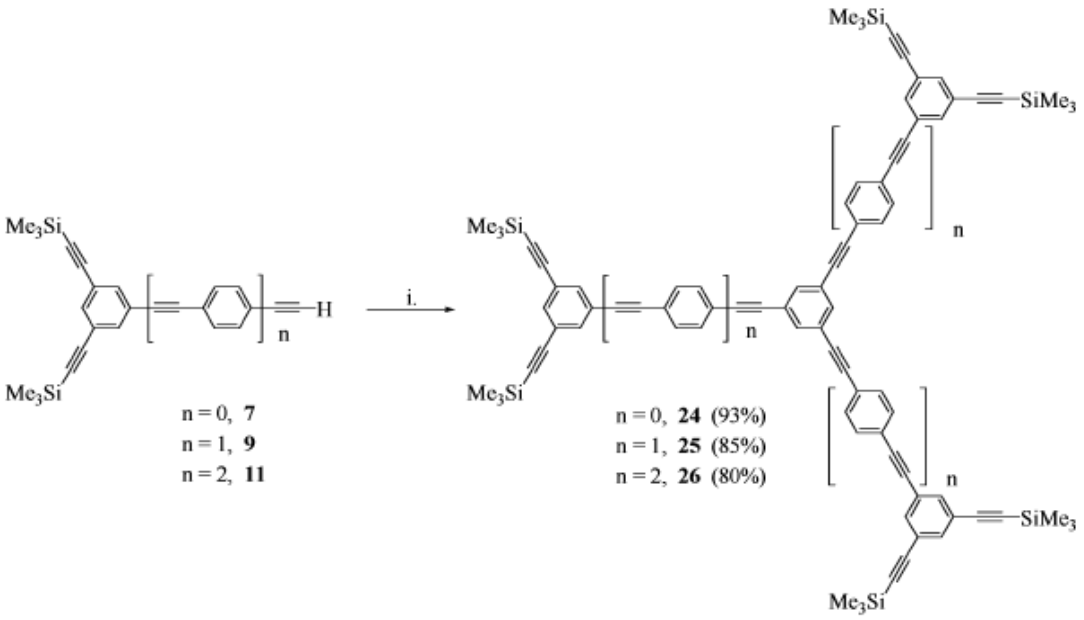


Figure 3

The study of their photophysical properties evidenced, that the maximum absorption wavelength in the UV-vis spectra (figure 4), had a bathochromic shift consistent with the increased conjugated phenylene ethynylene number *n* of repeated units. The corresponding molar extinction coefficients also increased with *n*. All the ethynylphenylene homologue compounds obtained showed a fluorescence emission radiation, with a bathochromic shift of 20 nm for each additional ethynylphenyl unit increasing the conjugated chain. In general, the higher homologues showed high quantum yields (65-82%), which increased with conjugation.

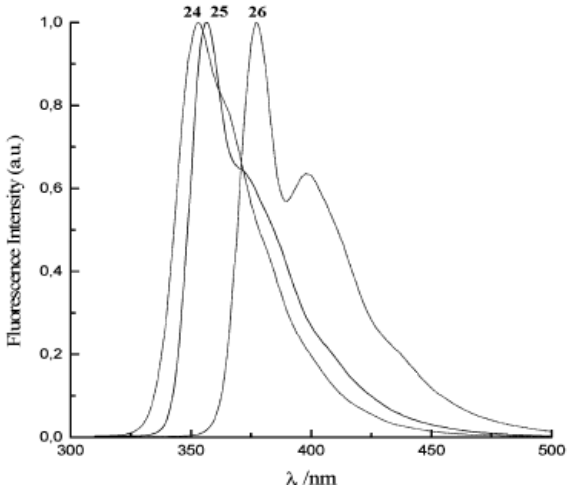


Figure 4: Normalized emission spectra in dicloromethane at RT.

Other appealing property of OPEs discovered corresponds to their ability to generate singlet oxygen ($^1O_2$). The study by Monnereau and Andraud, reported recently, highlighted this characteristic of the OPEs systems.[7]

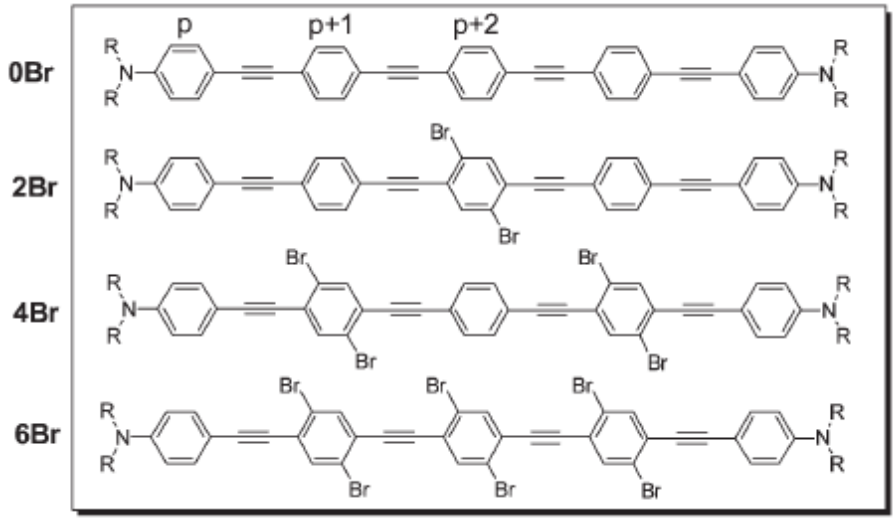


Figure 5

A series of compounds was obtained through a convergent "building-blocks" synthesis based on successive Sonogashira–Hagihara reactions. It was demonstrated by the detection of the characteristic $\Delta^1O_2 \rightarrow \Sigma^3O_2$ phosphorescence at 1270 nm, that all brominated compounds shown in Figure 5 (2Br, 4Br and 6Br) generated singlet oxygen, with quantum yields of 0.10, 0.50 and 0.35, respectively. Interestingly, fluorescence quantum yields and lifetimes evolved opposite to the $^1O_2$ generation quantum yield (Figure 6).

[7]Lanoë, P. H.; Gallavardin,T.; Dupin,A.; Maury, O.; Baldeck, P. L.; Lindgren, M.; Monnereau C.; Andraud, C.; *Org. Biomol. Chem.*, **2012**, 10, 6275-6278.

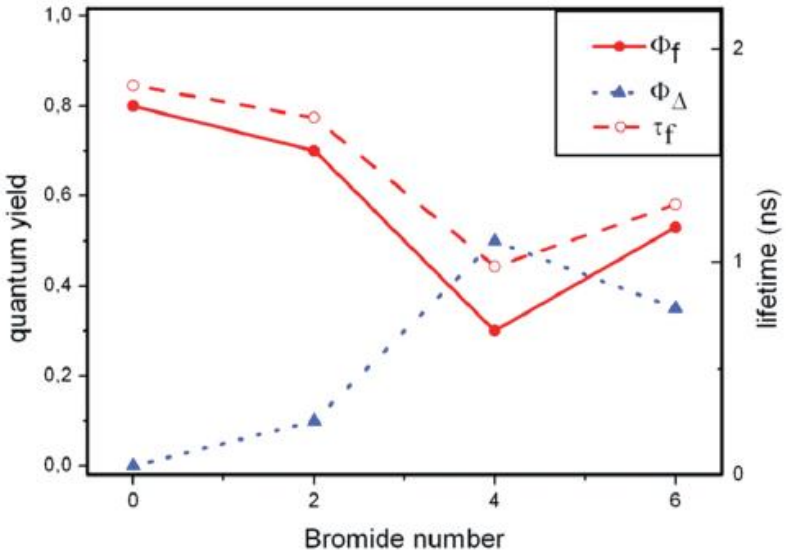


Figure 6: Evolution of the photophysical parameters (fluorescence quantum yield Φ_f ;$^1O_2$ generation quantum yieldΦ_Δ, lifetime τ_f) among the chromophores' series (298 K, $CHCl_3$).

This was a very interesting result because it gave the possibility to use OPEs as photosensitizers, and showed that these are molecules possessing an accessible triplet excited state able to generate singlet oxygen ($^1O_2$) by an energy transfer to ground state oxygen, ultimately leading to cell death.[8]

1.3. Aggregationof OPEs

The fundamental properties of many OPEs and PPEs, such as light absorption and emission, depends not only on their molecular structure but also on the supramolecular interactions and on the nano/mesoscale organization of the chains in the solid state. Pescitelli *et al.* investigated the aggregates formed in solution of proper solvents or solvent mixtures of two related glucose-substituted OPEs and PPEs shown in Figure 7: the oligomer HO (n ≈ 10) and the alternate copolymer AP (n≈ 40, Figure 7).[9]

[8]DeRosa, M. C.; and Crutchley, R. J.; *Coord. Chem. Rev.*, **2002**, 233–234, 351–371

[9] Pescitelli, G., Omar,O. H., Operamolla,A., Farinola,G. M., Di Bari, L., *Macromolecules* **2012**, 45, 9626−9630

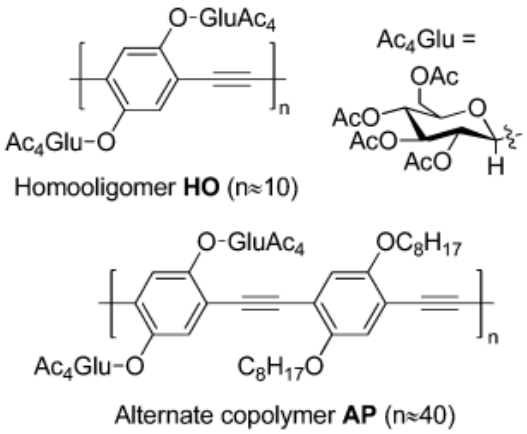


Figure 7

Formation of aggregates was detected by absorption and luminescence spectroscopies for the alternate copolymer AP and for the homo oligomer OH in chloroform/methanol (used as solvent/non solvent) mixtures or in chloroform. They could also evidence that both HO and AP remained quite emissive upon aggregation. In particular, the copolymer AP retained43% of its emission, which demonstrated that Glucose units represent efficient substituents for reducing the quenching of aggregated PPE, in addition to the properties of bulkiness and chirality.[10] It is noteworthy that in the 30:70 chloroform/methanol mixture only a small band of absorption was visible. In fact, in these conditions the few aggregates formed have a well defined structure which is preserved upon further aggregation. For the oligomer HO, the evolution of absorption spectra upon methanol addition is less evident than for the polymer AP (Figure 8). This was due to the presence of glucose groups that introduced some rigidity even in the oligomer, and prevented an effective aggregation.

[10] Zahn, S.; Swager, T. M. *Angew. Chem., Int. Ed.***2002**, 41, 4225−4230.

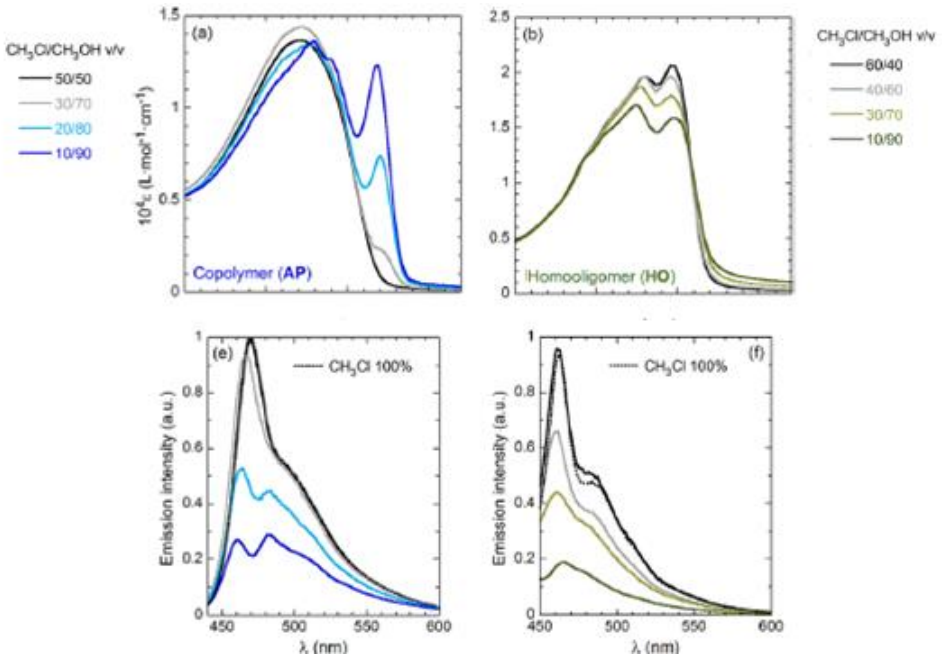


Figure 8. UV−vis absorption (top), and fluorescence (bottom) spectra of PPEs AP (left) and HO (right) in chloroform/methanol mixtures, normalized per monomer (9 × 10−5 M; 1 cm cell).

Fernández *et al.*reported in 2013 the synthesis of the new oligophenylene ethynylene (OPE) Palladium derivative represented in figure 9,and investigated its self-assembly in solution, in the bulk state, and on surfaces.[11]The performed experiments to determine how the OPE compound aggregates were temperature and concentration dependent.

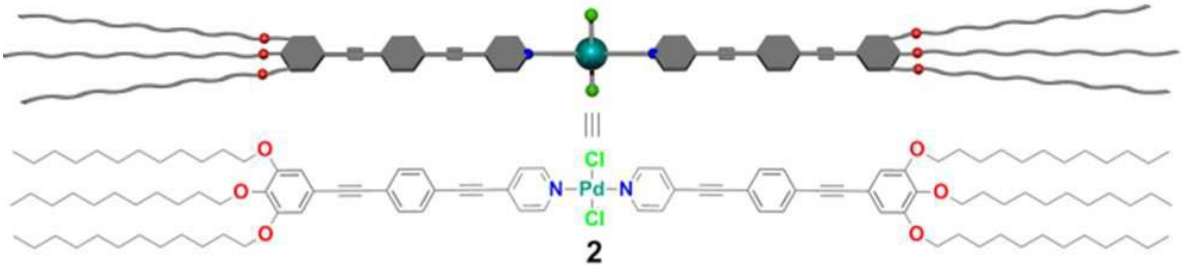


Figure 9

[11] Mayoral, M. J.;Rest,C.; Stepanenko,V., Schellheimer, J.; Albuquerque, R.;Fernández G., *J. Am. Chem. Soc.***2013**, 135, 2148−2151

In fact, solutions of compound **2** in 6-mercapto-1-hexanol (MCH) at different concentrations were slowly cooled down from 343 to 278 K at a rate of 0.5 K min^{-1} to ensure that the process took place under thermodynamic control. Above 313 K, **2**exhibited a sharp transition with a maximum at λ~350 nm that could be assigned to a molecularly dissolved state.[12] On cooling the solution, a new red-shifted transition centered at λ=371 nm was noticed that spreads into the visible region up toλ~470 nm (Figure 10). This behavior could be attributed to the formation of self-assembled structures.

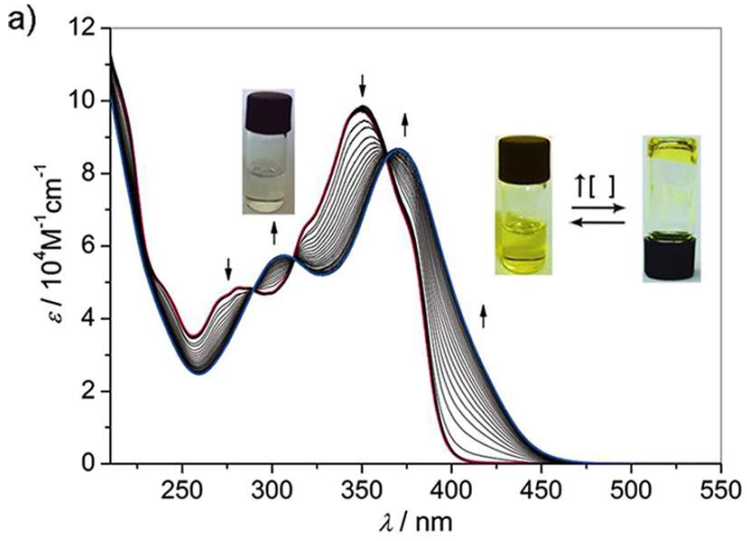


Figure 10

1.4.Applications of OPEs in biology

There are very few examples of application of OPEs in the biological field. Among them, interesting examples were illustrated by the work of Whitten *et al.*,[13]which evidenced that the presence of a proper functionalization was essential to be used in the biological medium. Thus,

[12]a) Chen, Z.; Lohr, A.; Saha-Möller, C. R.; Würthner, F. *Chem. Soc. Rev.***2009**, 38, 564; b) De Greef, T. F. A.; Smulders, M. M. J.; Wolffs, M.; Schenning, A. P. H. J.; Sijbesma, R. P.; Meijer, *E. W. Chem. Rev.***2009**, 109, 5687-5754.

[13]a) Hill, E. H.; Evans, D. G.; Whitten, D. G. *Langmuir***2013**, *29*, 9712−9720. b) Dascier, D.; Ji, E.; Parthasarathy, A.; Schanze, K. S.; Whitten, D. G. *Langmuir* **2012**, *28*, 11286−11290.

the structure depicted in Figure 11, functionalized with two tetraalkylammonium side groups, was soluble in water and exhibited dark and light-activated biocidal activity. This water soluble family of conjugated oligomers had affinity for bacterial cell membranes and was shown to cause membrane disruption, agglomeration, and bacterial death.

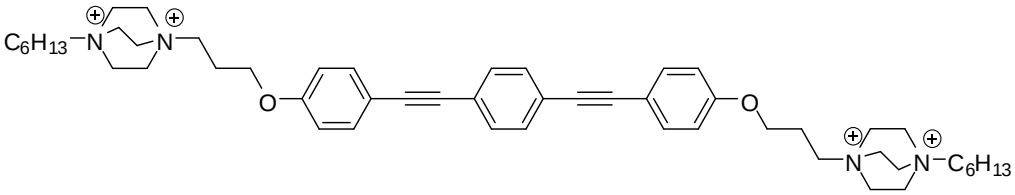


Figure 11

Moreover, this compound had a remarkable bactericidal activity, and was demonstrated to be very effective against *Escherichia coli (E. coli)* biofilms when irradiated with UV light (Figure 12). The damage was largely caused by the production of singlet oxygen by the OPEs when irradiated. A broad-spectrum damage to nucleic acids and proteins in the cell was produced.

Figure 12

The ultimate aim of this investigation was the use of cationic end-only functionalized oligo(arylene-ethynylene)s(EO-OPEs) for preventing and eliminating *Escherichia coli (E.coli)* infections in hospital and community acquired, to health care systems.

Functionalized oligo(phenylene-ethynylene)s (OPEs) with different conjugation lengths were used as luminescent labeling probes for proteins: their synthesis, reported by the group of Che,[14] utilized the Sonogashira coupling as key step. The photoluminescent properties for the OPE series with different chain lengths and their solvatochromic responses were examined. Introduction of a *N*-hydroxysuccinimidyl (NHS) moiety into the oligo(arylene-ethynylene)s, as indicated in Figure 13, enabled covalent attachment of the fluorophore to the protein HSA (Human serum albumin) though the substitution of the *N*-hydroxysuccinimideby a lysine residue of the protein. This substitution was possible to the known ability of the primary NH_2 of lysine to react with *N*-hydroxysuccinimide moiety .

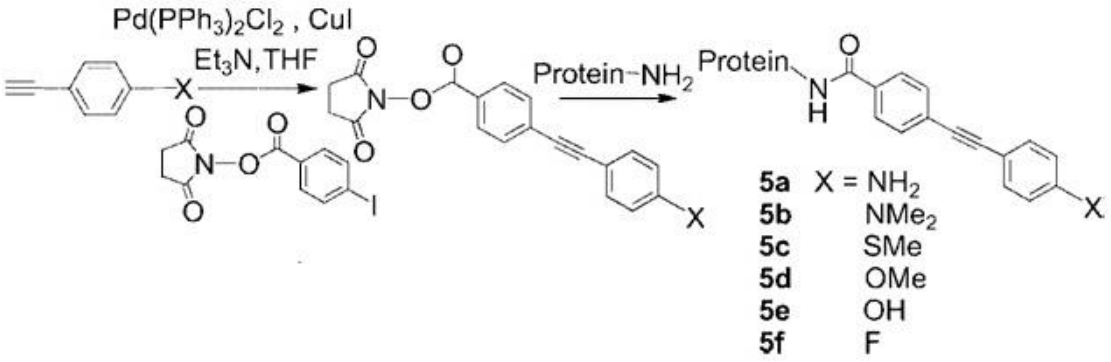


Figure 13

In 2013, the synthesis of a new class of rigid ethynyl terminated OPE spacers of variable length[15]was described by Bernardi *et al* .The copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) "click" reaction of these spacers with glycosil azides (Figure 14) led to the formation of sugar terminated OPEs: the work was projected with the intent of testing the activity of the

[14]Zhi, Y.-G.; Lai, S.-W.; Chan, Q. K.-W.; Law, Y.-C.; Tong, G., S.-M.; Che, C.-M. *Eur. J. Org. Chem.* **2006,** 3125−3139.

[15]Pertici, F., Varga, N.; van Duijn, A.; Rey-Carrizo, M.; Bernardi, A., Pieters, R. J.; *Beilstein J. Org. Chem.* **2013,** *9*, 215–222.

synthesized oligomers on bacterial adhesion inhibition. As the target, the bacterial lectin LecA, a virulence factor of the problematic pathogen *Pseudomonas aeruginosa*, was chosen. Knowing that this lectin was able to bind galactosides and that the shortest distance between two binding sites is around 26 Å, the three-unit OPE spacer represented in Figure 14, measuring around 22 Å without the aglycon linking moiety, was chosen for this experiment.

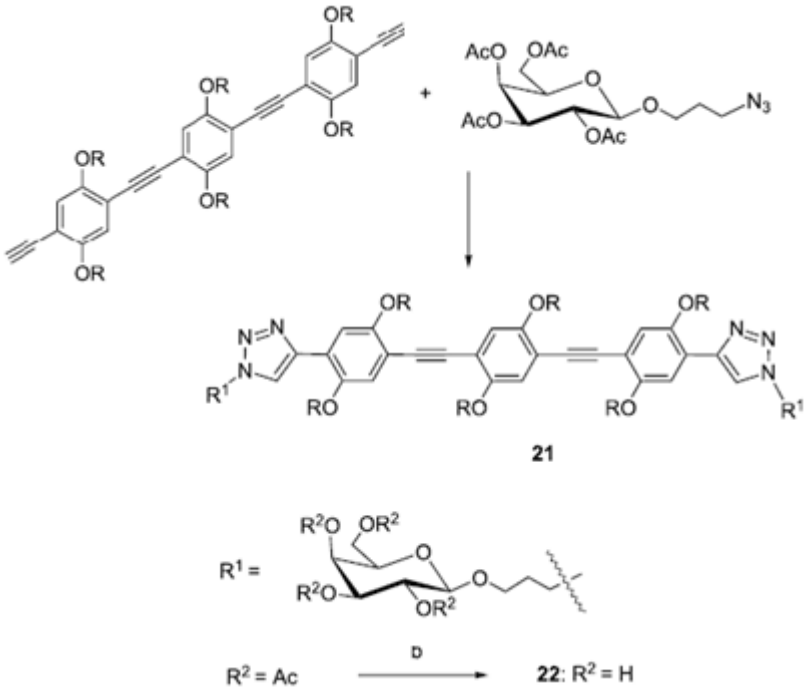


Figure 14

In an ELISA (enzyme-linked immunosorbent) type assay, the behavior of deprotected carbohydrate derived compound **22** as inhibitor against the lectin LecA from *Pseudomonas aeruginosa* was demonstrated.

In 2015, Bernardi *et al.*reported the design of other glycoside substituted OPEswith the aim of finding inhibitors of HIV infection,[16] particularly targeting against DC-SIGN, a tetrameric C-

[16]Ordanini,S.; Varga, N.; Porkolab, V.; The´paut, M.; Belvisi, L., Bertaglia, A.; Palmioli, A., Berzi, A.; Trabattoni, D.; Clerici, M.; Fieschi, F.; Bernardi, A. *Chem. Commun.*,2015, 51, 3816-3819.

type lectin receptor of dendritic cells involved in many pathogens infection cycle (HIV, Ebola and Dengue).The rod-like rigid OPE spacers were linked to multivalent glycosidic ligands by exploiting click-chemistry (Figure 15). A range of selected compounds were tested in a sensitive assay, using a cellular model of HIV-1 infection. The results of these transinfection studies showed a clear dose–response effect for all the tested inhibitors.

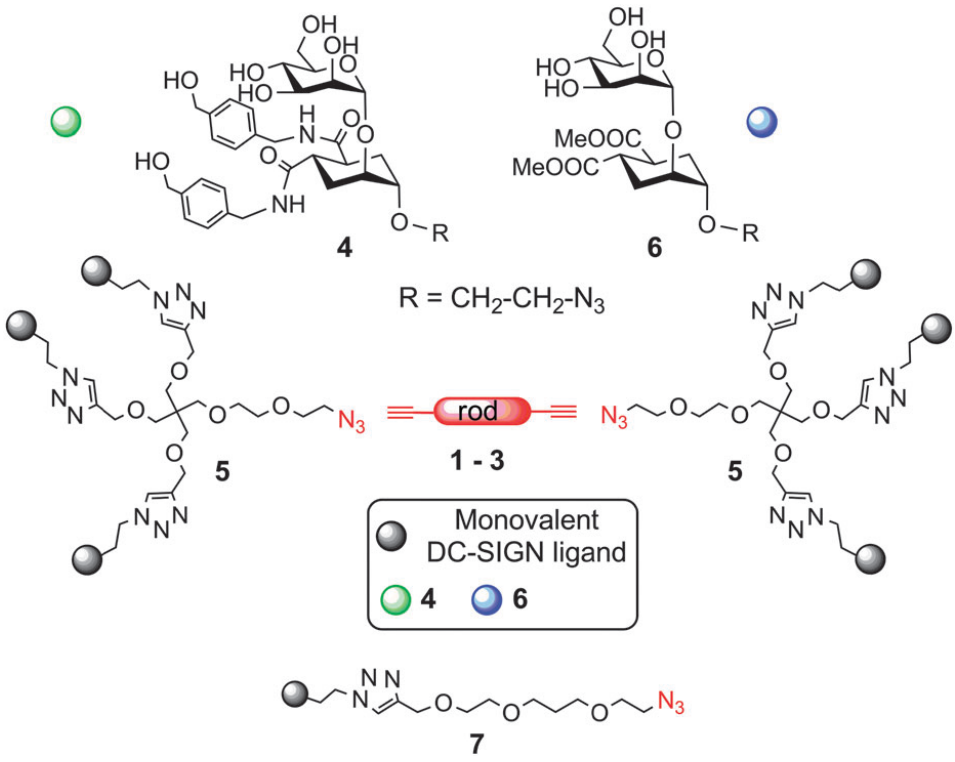


Figure 15

The only example of the use of an OPE as a cell dye, was described by Farinola in 2012.[17] The amphiphilic aryleneethynylene O1 derivative represented in Figure 16 was used as a plasma membrane marker in fixed and living mammalian cells and liposome model systems,The structure included a central fluorene and two phenyleneethynylene branches to ensure good fluorescent properties (O1, figure16). Moreover, alkyl chains of 18 carbon atoms were

[17] Cardone, A.; Lopez, F.; Affortunato, F.; Busco, G.; Hofer, A. M.; Mallamaci, R.; Martinelli, C.; Colella, M.; Farinola, G. M. *Biochimica et Biophysica Acta***2012***1818*, 2808–2817.

introduced as alkoxy groups on the external phenyl rings, and polar sulfonate groups were attached to the central fluorene ring to guarantee the amphiphilic character of O1. The synthesis of the compound O1 was based on a convergent palladium-catalyzed cross-coupling. O1 exhibited fast staining, insensitivity to pH in the patho-physiological range, good photostability and effective multicolor labeling in inmunofluorescence experiments.

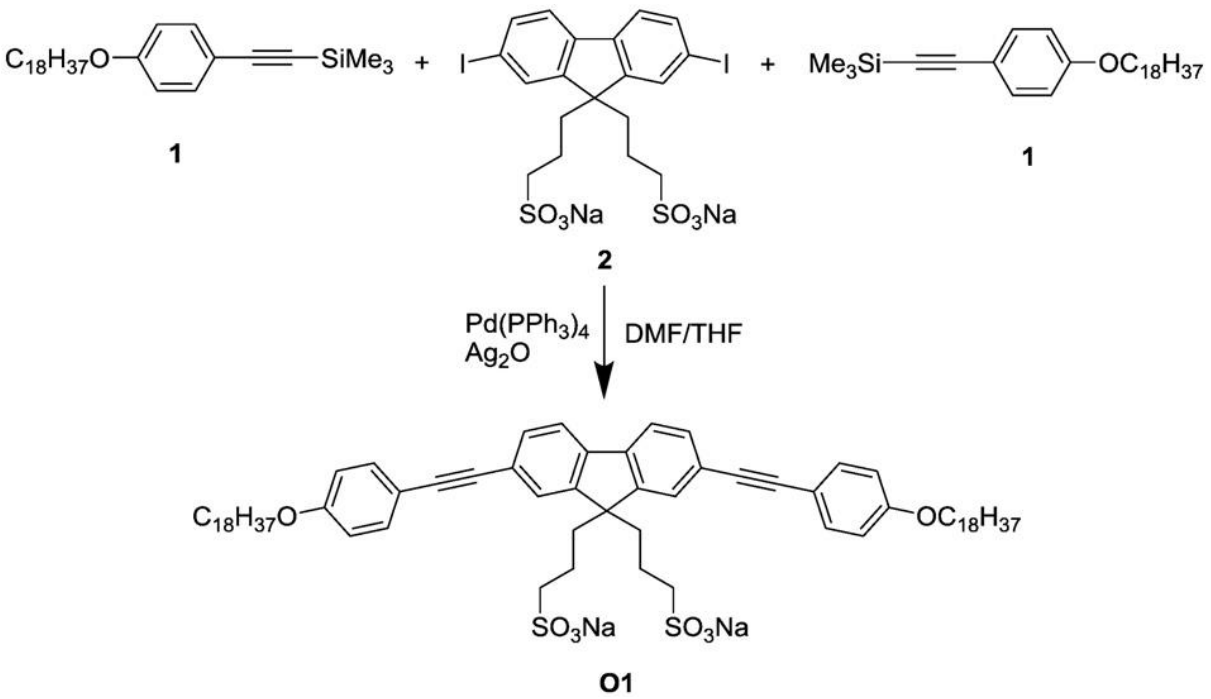


Figure 16

The reported results confirmed that these luminescent molecules, if opportunely functionalized, can be conveniently used as a novel class of membrane markers for a wide range of biological applications.

1.5. Objectives

Considering the precedent work, and taking into account the expertise of the research group at the Universitá di Messina in the chemistry of carbohydrates,[18] we decided to focus in the synthesis of differently substituted Oligo(Phenylene) Ethynylene (OPE) systems incorporating two glycosides at the external units. The presence of monosacharides in the OPE structure would add to the conjugated systems two natural fragments which could extend the applications of these synthetic molecules in the biological field by increasing their biocompatibility. This could facilitate for instance, the use of the resulting glycosilated OPEs as fluorescent probes in biological processes. Another important advantage of the presence of monosacharides in the OPEs would be the increase of their solubility in water expected in the sugar containing final targets. Looking for useful applications, a versatile synthesis was required, since the possibility of making products with different relative ratios of π-conjugated fragments (hydrophobic) versus carbohydrates (hydrophilic) would also be essential.

Taking into account all these considerations, we envisaged the following objectives for this Ph D work:

1.5.1 Synthesis and properties of glucosyl derived OPEs

Synthesis of 1,4-dimethoxyphenyl-2,5-ethynylene oligomers. A series of 1,4-dimethoxyphenyl-2,5-ethynylenes derivatives with up to three units, incorporating two propargyl glucosides at

[18] (a) Bonaccorsi, P.; Di Gioia, M. L.; Leggio, A.; Minuti, L.; Papalia, T.; Siciliano, C.; Temperini, A.; Barattucci, A. *Beilstein J. Org.Chem*. **2013**, *9*, 2410−2416. (b) Bonaccorsi, P.; Aversa, M. C.; Barattucci, A.; Papalia, T.; Puntoriero, F.; Campagna, S. *Chem. Commun*. **2012**, *48*, 10550−10552. (c) Bonaccorsi, P.; Marino-Merlo, F.; Barattucci, A.; Battaglia, G.; Papaianni, E.; Papalia, T.; Aversa, M. C.; Mastino, A. *Bioorg. Med. Chem.* **2012**, *20*, 3186−3195. (d) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P. Synlett **2011**, 254−258. (e) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P.; Temperini, A. *Eur. J. Org. Chem.* **2011**, 5668−5673. (f) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P.; Marino-Merlo, F.; Mastino, A.; Sciortino, M. T. Bioorg. Med. Chem. 2009, 17, 1456−1463. (g) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P. *Eur. J. Org. Chem.* **2009**, 6335−6339. (h) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P. *Tetrahedron* **2008**, *64*, 7659−7683. (i) Aversa, M. C.; Barattucci, A.; Bilardo, M. C.; Bonaccorsi, P.; Giannetto, P.; Rollin, P.; Tatibouet, A. *J. Org. Chem.* **2005**, *70*, 7389−7396.

the terminal moieties, was initially proposed. These target structurers **A**-**C** are indicated in Figure 17.

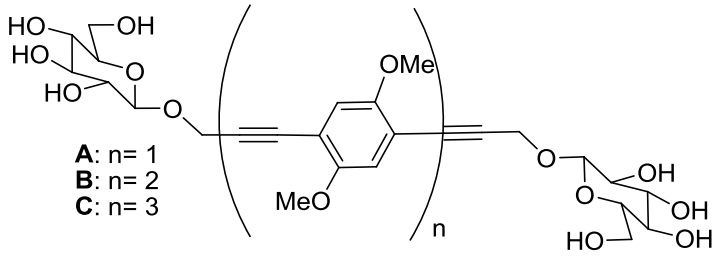
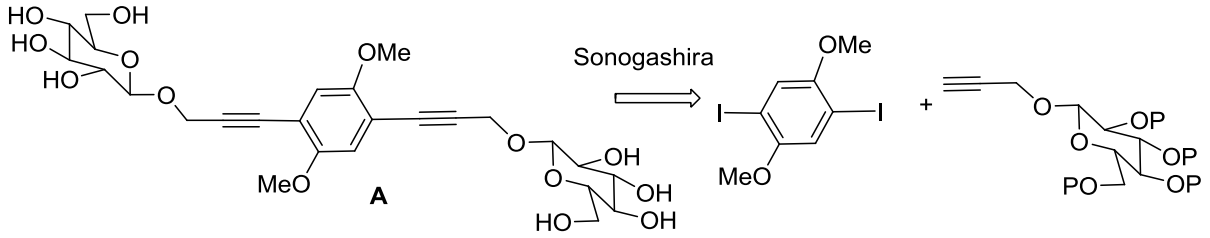


Figure 17

The retrosynthesis that would allow the rapid construction of these skeletons was based on the Pd catalyzed Sonogashira coupling of 2,5-dimethoxy-1,4-iodobenzene or adequately substituted analogues, with functionalized alkynes. This is illustrated for the monomer **A** in Scheme 1. The known propargyl glucoside, with all the OH groups protected, will be used for this coupling, to introduce the glucoside. After the Sonogashira type reaction, deprotection of the carbohydrate would produce the the desired substrate.



Scheme 1

Synthesis of OPE glucosides with benzene and N,N-dimethyl aniline cores.

The structures represented in Figure 18 would also be synthesized using a similar strategy. These are characterized by the presence of different substituents in the central core of the OPEs, such a benzene (**D**), one or two N,N-dimethyl aniline rings (**E** and **F**) and the corresponding ammonium salts (**G** and **H**).

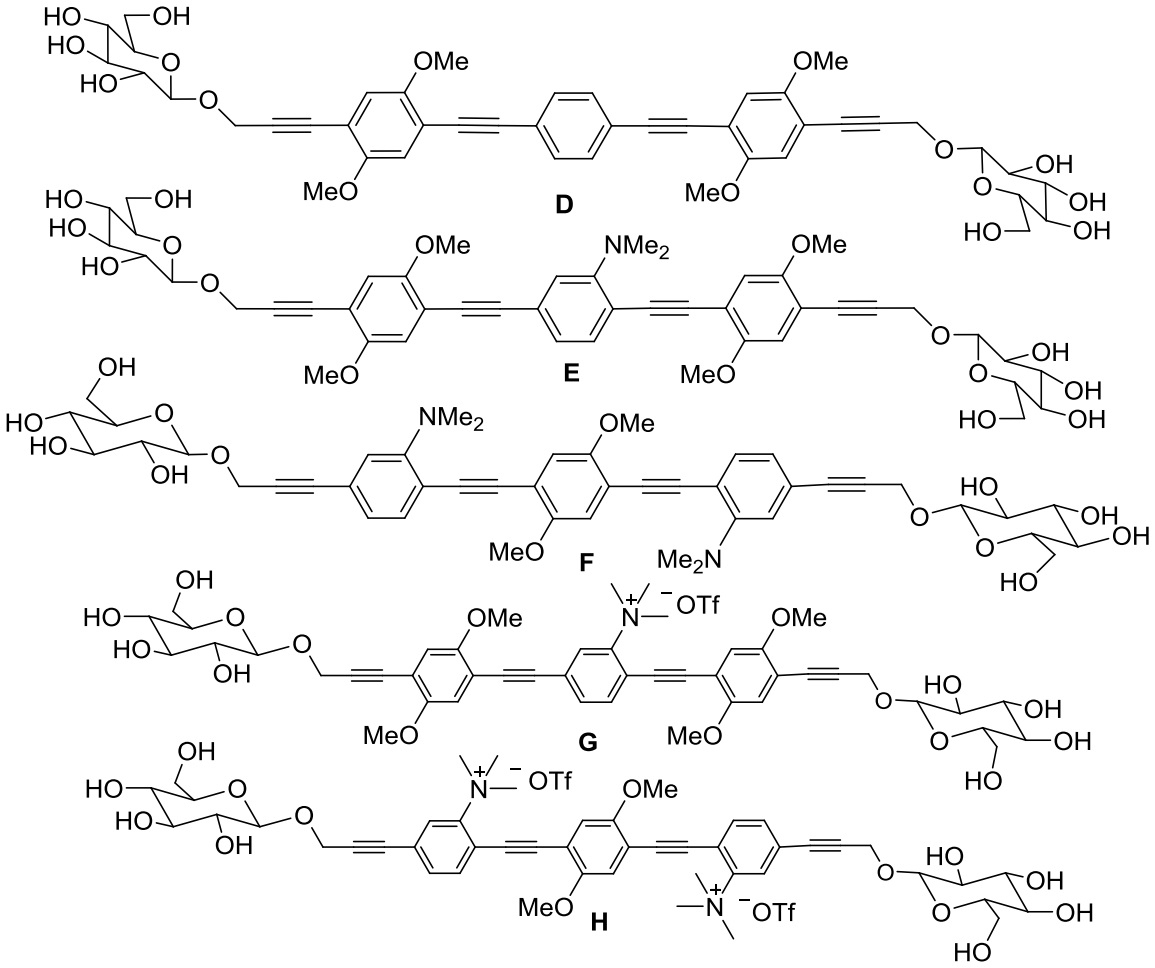


Figure 18

Photo-physical measurements and singlet oxygen evaluation.

With these structures in hands, a study of photophysical properties, using UV/vis and luminiscense measures, would be undertaken.

Biological studies.

The biological applications of these glucosides OPEs required a study of the behavior of these molecules faced to living cells. A primary aspect that was investigated was the possibility of the synthesized derivatives to go through the cell membranes.

Cell internalization.

The ability of the OPE glucosides to enter into the cells was studied to evaluate the possibility of further applications within the biological field.

Behavior of OPEs as photosensitizers in PDT.

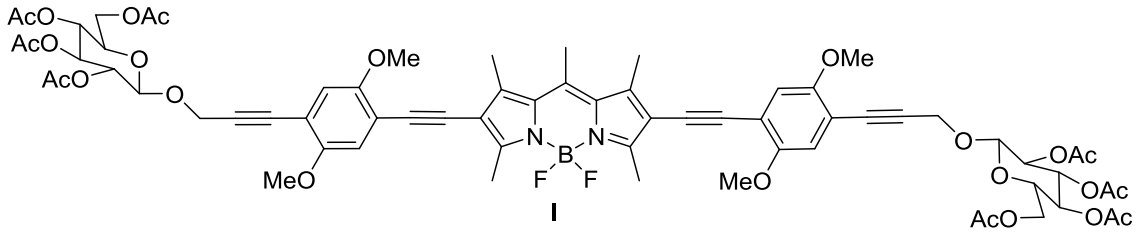
To investigate the possibility of using the synthesized OPEs as photosensitizers in Photodinamic Therapy other aspects of the behavior of these molecules face to the living cells must be initially studied. The efficiency of a photosensitizer for PDT is related to its ability to produce singlet oxygen under irradiation as well as to its subcellular localization.[19] Both the ability to produce $^1O_2$ under irradiation and the cell internalization and subcellular localization of OPEs **18** and **28** in HaCaT (human keratinocytecells), HeLa (human cervical cancercells) and HEp-2 (human larynx cancer cells) cells wereanalyzed by fluorescence microscopy under UV excitation light.

1.5.2. Synthesis of BODIPY-OPEs containing glucosides

BoronDiPyrromethene (BODIPY) chromophores are highly fluorescent molecules[20] which could considerably shift the wavelength of adsorption and emission of different molecules. Some glycoconjugated BODIPY derivatives[21] have been synthesized in Messina to be applied in bio-imaging.[22] In order to move further into the biological applications of our work, we thought of introducing astructural modifications on our OPEs, which could lead to a shift of the absorption and emission to higher wavelengts, near the biological 'red-window'. The presence of a BODIPY moiety into our OPE glucosides, would probably improve their photophisycal properties for further applications. The interest of synthesizing a BODIPY containing structure such as **I**, indicated in Figure 19, stemmed on the expected improvement of the luminescent properties due to the presence of the heterocyclic core. This could open the

[19](a) Soares, A.R.M.; Neves M.G.P.M.S.; Tome, A.C.; Iglesias-de la Cruz M.C.; Zamarrón, A.; Carrasco, E.; González, S.; Cavaleiro, J.A.S.; Torres, T.; Guldi, D.M.; Juarranz, A., *Chem. Res. Toxicol.* **2012**, *25*, 940-951. (b) Rello-Varona, S.;Gámez, A.;Moreno, V.;Stockert, J.C.; Cristóbal, J.;Pacheco, M.; Cañete, M.; Juarranz, A.; Villanueva, A. , *Int. J. Biochem. Cell. Biol*. **2006**, *38*, 2183–2195.
[20]Ulrich, G.; Ziessel, R.; Harriman, A.;*Ang. Chem. Int. Ed.* **2008**, *47*, 1184-1201
[21]Papalia, T.; Barattucci, A.; Aversa, M. C.;Campagna, S.; Puntoriero, F.; Bonaccorsi, P.; *Chem. Commun.***2012**, 48, 10550–10552
[22]Papalia, T.; Siracusano, G.; Colao, I.; Barattucci, A.; Aversa, M. C.;Serroni, S.; Zappala, G.; Campagna, S.; Sciortino M. T.; Puntoriero, F.; Bonaccorsi, P.; *Dyes Pigments***2014**, 110, 67.

way to more efficient systems. Moreover, a good affinity of **I** for the cells could be expected due to the presence of the glucose fragments. We thus proposed compound **I** as the next target, since we envisaged that its synthesis could be completed, once again,using a similar strategy based on the use of Sonogashira reaction.



I

Figure 19

1.5.3. Synthesis of AZO-OPEs containing glucosides

Other interesting core that was considered for introduction into the OPEs was the azobenzene. Azobenzenesare a group of compounds with the capacity to adsorb energy and use it for a molecular movement: the cis-trans isomerization. This process is a reversible transformation between two geometrical isomers leading to a notable change in the photophysical properties of the azobenzene, particularly in the dipolar moment and in the absorption spectrum.[23]

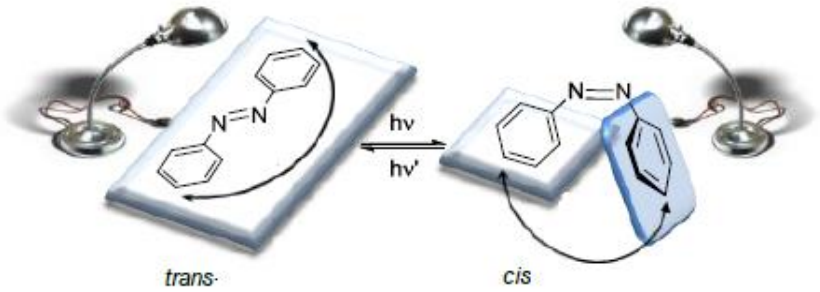


Figure 20

In previous work, the research group at the *Universidad Autónoma de Madrid* studied the photoisomerization and photochromic properties of different enantiopure sulfinyl azobenzenes

[23]a) Rau, H.; *Photocromism, Molecules and Systems*; **1990**, 165. b) Suginome, H.; *CRC Handbook of Organic Photochemistry and Photobiology*; **1995**, 824.

evidencing an efficient transfer of chirality from the sulfoxide to the azo compound both in the cis and trans isomers which is controlled by the chirality at the sulfur atom.[24]The idea of synthesizing an OPE derivative with an azobenzene core was appealing in order to study how the photophysical properties of the resulting OPE were modulated by the azo group upon *cis-trans* photoisomerization . The presence of the glucosides in compound J, would make it biocompatible thus, opening the way for new biological applications.

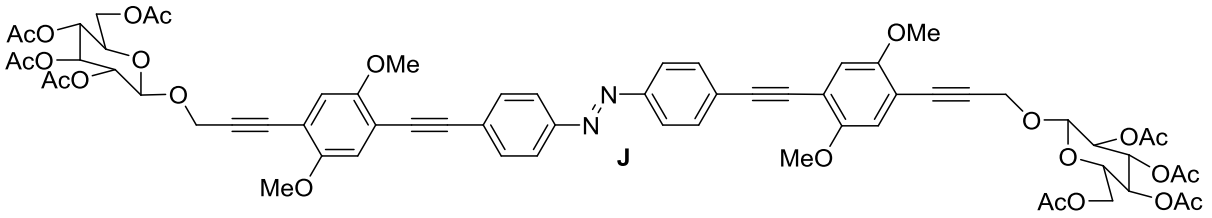
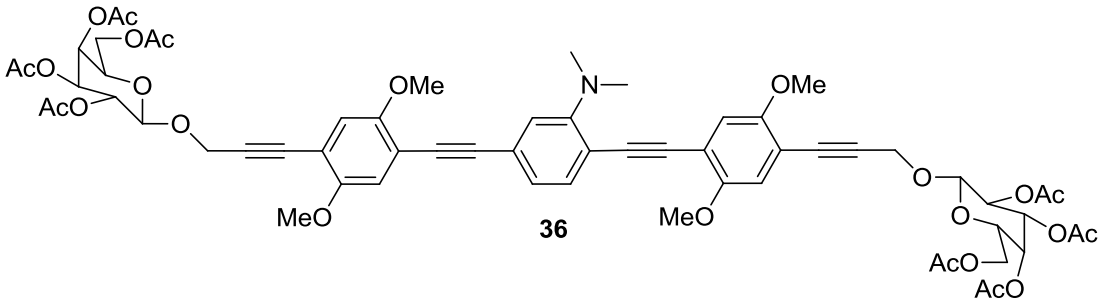


Figure 21

1.5.4. Synthesis of galactose derived OPEs

Taking into account that a different monosaccharide could change the biological activity of the projected OPES, galactose containing OPEs would also be synthesized using a similar strategy.

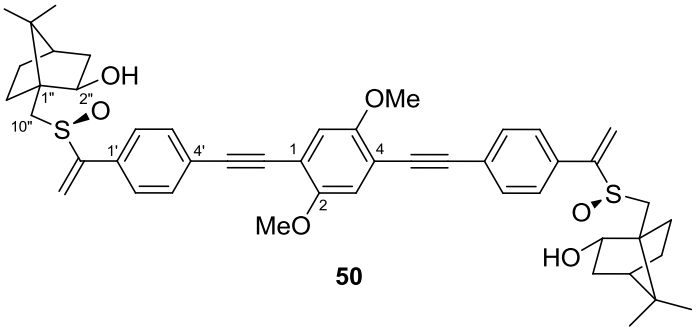


[24]a) I.Núñez, E. Merino, M. Lecea, S.Pieraccini, G. P.Spada, C.Rosini, G.Mazzeo, M.Ribagorda, M. C.Carreño, *Chem.Eur. J.***2013**, *19*, 3397-3406. b) M. C. Carreño, I. García, I. Núñez, E. Merino, M. Ribagorda, S. Pieraccini, G. P. Spada*J. Am. Chem. Soc*. **2007**, *129*, 7089-7100; c) M. C. Carreño, I. García, M. Ribagorda, E. Merino, S. Pieraccini, G. P. Spada, *Org. Lett*. **2005**, *7*, 2869-2872; d) M. C. Carreño, G. Fernández Mudarra, E. Merino, M. Ribagorda, *J. Org. Chem*. **2004**, *69*, 3413-3416.

Figure 22

1.5.5. Synthesis of sulfoxide derived OPEs.

The research groups of Messina[25] and Madrid[26] have a great experience on stereoselective synthesis monitored by sulfoxides. The possibility of using phenylene ethylene units in the construction of C2-symmetric molecular architectures containing enantiomerically pure sulfinyl functions as potential chelating agents would be another interesting goal. In particular, the isoborneol sulfinyl moiety appears to be a significant chiral controller and an interesting _structurally privileged residue. For this purpose, the combining of the classical synthetic procedure of OPE preparation, the Sonogashira cross-coupling, with the well-known sulfenic acid/alkyne syn-addition to obtain sulfinyl derivatives in a stereocontrolled manner could represent an efficient strategy to builds sulfinyl OPEs as 50.



50

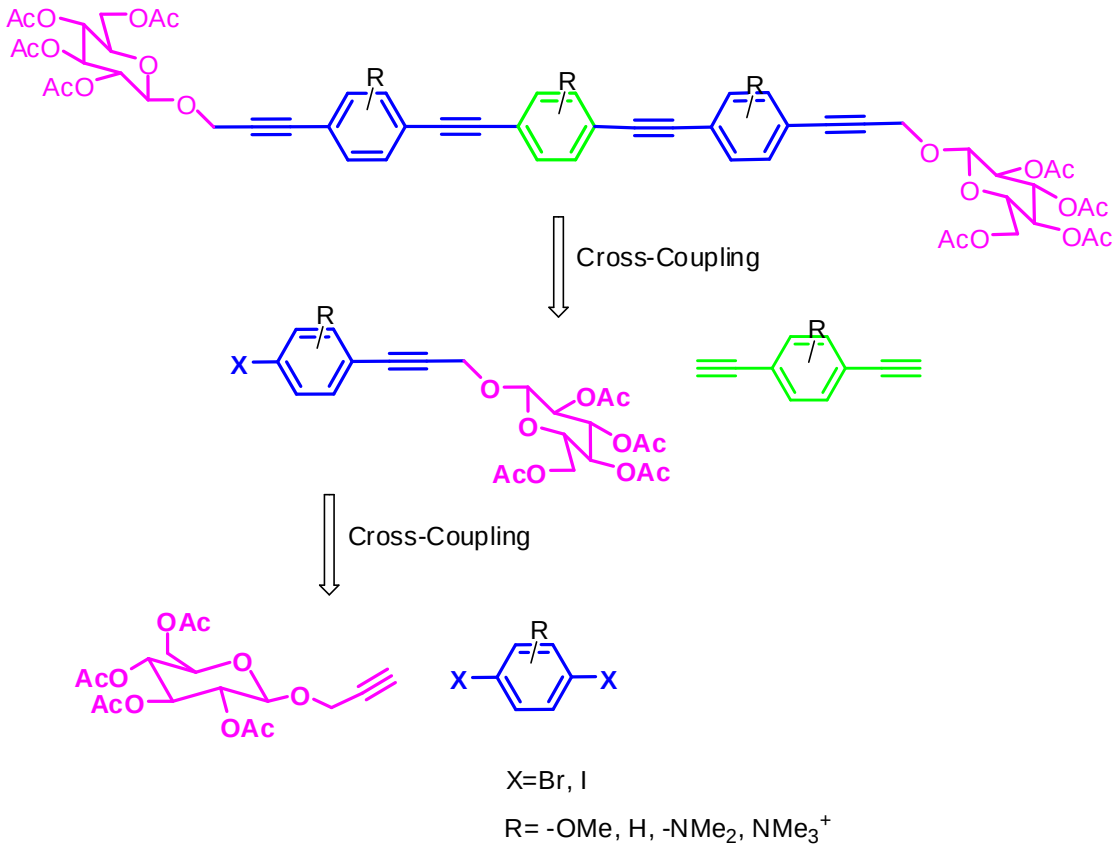
[25] a) Barattucci, A.; Di Gioia, M.L.; Leggio, A.; Minuti, L.; Papalia, T.; Siciliano, C.; Temperini, A.; Bonaccorsi, P. *Eur. J. Org. Chem.* **2014**, 2099; b) Barattucci, A.; Plutino, M.R.; Faggi, C.; Bonaccorsi, P.; Monsu` Scolaro, L.; Aversa, M. C. *Eur. J. Inorg. Chem.* **2013**, 3412; c) Bonaccorsi, P.; Marino-Merlo, F.; Barattucci, A.; Battaglia, G.; Papaianni, E.; Papalia, T.; Aversa, M.C.; Mastino, A. *Bioorg. Med. Chem.* **2012**, *20*, 318: d) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P.; Faggi, C.; Gacs-Baitz, E.; Marrocchi, A.; Minuti, L.;Taticchi, A. *Tetrahedron* **2005**, *61*, 7719.

[26] For a review, see: A. Urbano, M. C. Carreño, *Or.g Biom. Chem.* **2013**, *11*, 699-708A. For recent work see: a) A. M. del Hoyo, A. Latorre, R. Díaz, A. Urbano, M. C. Carreño; *Adv. Synt. Cat.* **2015**, 357, 1154-1160. b) Latorre, A. Urbano, M. C. Carreño, *Chem.Comm.* **2011**, *47*, 8103–8105. c) A. Latorre, A. Urbano, M. C. Carreño, *Chem. Commun.*, **2009**, 6652-6655

Synthesis And Properties Of Glucosyl Derived OPEs

Chapter 2

The synthesis of the oligo phenylene ethynylenes that we had proposed as initial target structures for this PhD work, was achieved following the retrosynthetic scheme shown below.



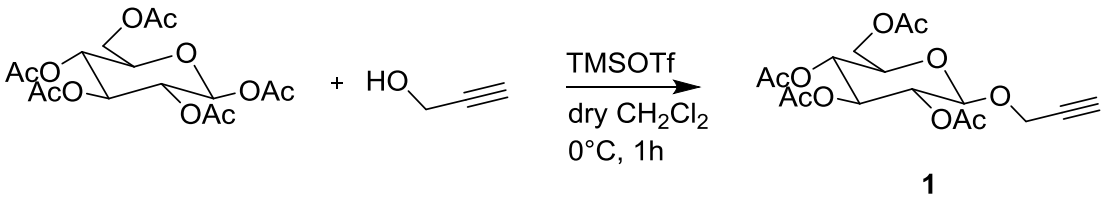
The methodology applied for the building of our OPEs structures is based on the iterative use of Pd-mediated Sonogashira reaction. By this way, we can couple the glucosidic fragment to an bromo or iodo substituted aromatic ring and then link, the obtained monoderived compound, to a properly substituted central core.

The methodoly is very generic, in fact, it was applied to the synthesis of a wide range of different OPEs, obtained only synthesized the appropriate aromatic central brick.

2.1 Synthesis of 1,4-dimethoxyphenyl-2,5-ethynylene oligomers

After a careful research in the literature we could deduce that the most effective method to construct the skeleton of the conjugated oligomers object of this thesis is based on cross-coupling Sonogashira reactions. [1] Thanks to the use of this versatile synthetic method, it was possible to build a new family of oligomers.

In agreement with the retrosynthesis presented above, the first step was the choice and subsequent synthesis of the suitable building blocks to be later treated under the key Sonogashira conditions. The chosen starting products were 2-propynyl-O-*β*-glucopyranoside-2,3,4,6-tetraacetate **1** and 2,5-dimethoxy benzene **2** (schemes 1 and 2 respectively). Although **1** was commercially available, it was synthesized in the laboratory following a known procedure.[2] Thus,reaction between 2-propynyl-O-*β*-glucopyranoside-2,3,4,6-tetraacetate and propargyl alcohol in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) (Scheme 1) followed by purification by column chromathography led to **1** with high purity grade and in good yields (78%).



1

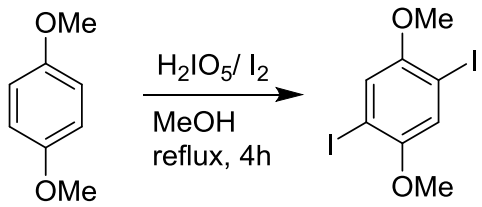
Scheme 1

[1] Chinchilla, R.; Nájera, C.; *Chem. Rev.***2007**, *107*, 874–922

[2] Giovenzana, G. B.; Lay, L.; Monti, D.; Palmisano, G.; Panza, L. *Tetrahedron* **1999**, *55*, 14123-14136.

The control of the reaction was really essential for its full success: in fact, a longer period than 1,5 hours caused considerable decrease in the yield percentage, because of the decomposition of the glucoderived alkyne **1** in the reaction conditions once formed.

The second precursor, 1,4-diido-2,5-dimethoxy benzene (**2**), was synthesized from commercially available 2,5-dimethoxy benzene by treatment with H_2IO_5 and I_2 in MeOH at 50°[3]. After 4h under vigorous stirring, the quantitative and unique formation of di-substitution product **2** was observed by TLC. Compound 2 was pure enough to be used in next step without further purification, (Scheme 2).



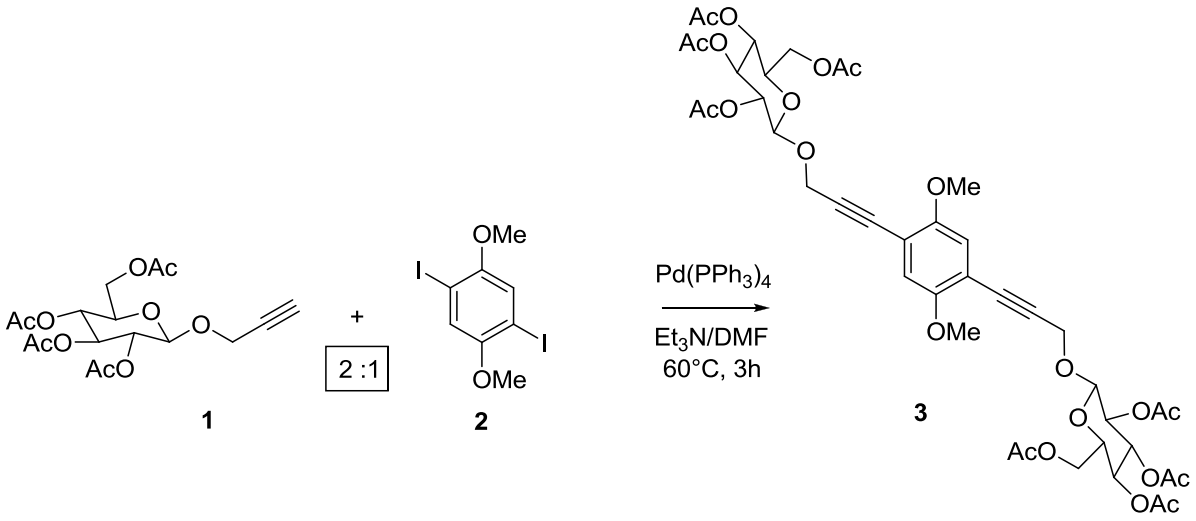
2

Scheme 2

The subsequent step was the assembly of the first conjugated system, using the two building blocks **1** and **2** previously synthesized, with the exploitation of a **Sonogashira** reaction, in the copper-free variant. In this cross-coupling reaction, Pd(0) catalyzes the carbon-carbon bond formation between a terminal alkyne and aryl or vinyl halides, leading to the synthesis of aryl or vinyl substitued alkynes. The reaction can occur with or without co-catalyst. In the first case, a co-catalyst (generally CuI) is used to generate *in situ* Pd(0) from Pd(II) salts. In the second case, the direct use of a Pd(0) complex could produce low yields, probably by oxidation of the metal

[3] Yi, C.; Blum, C.; Lehamann, M.; Keller, S.; Liu, S. X.; Frei, G.; Neels, A.; Hauser, J.; Schürch, S.; Decurtins, S. *J. Org. Chem.* **2010**, *75*, 3350-3357.

in the presence of atmospheric oxygen, if the conditions of reaction are not strictly degassed and dry. The use of a co-catalyst is in principle recommended, but there are some cases in which side reactions are produced in the presence of CuI. When we effected the reaction between compounds **1** and **2** in DMF using $Pd(PPh_3)_4$ as catalyst and CuI as a co-catalyst, we observed decomposition/auto-coupling of compound **1**. These undesired processes had been already reported in the literature[4]. After an accurate bibliographic research, it was clear that only the direct use of Pd(0) would allow a successfull cross-coupling reactions with glucosydic substrates.[5]



Scheme 3

We thus carried out the reaction between propargyl glucoside **1** and 1,4-diido-2,5-dimethoxy benzene **2** (in ratio 2:1) in a mixture 1:1 DMF/Et_3N, in presence of $Pd(PPh_3)_4$, under Argon atmosphere. The amount of catalyst proved to be critical for the efficiency of the process. In fact, due to the difficult separation of the resulting product **3** from the reaction crude containing the catalyst, it was essential to look for the minimum amount that could guarantee high yields and, at

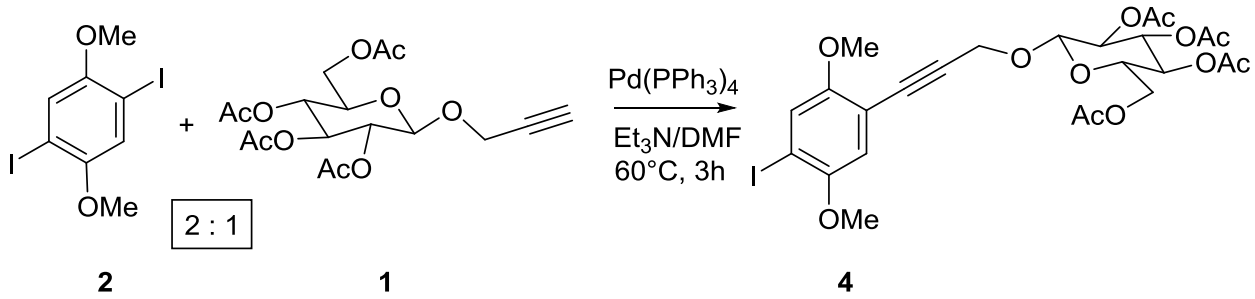
[4]André, S.; Liu, B.; Gabius, H. J.; Roy, R. *Org. Biomol. Chem*. **2003**, *1*, 3909-3916

[5] Roy, R.; Das, S. K.; Santoyo-González, F.; Hernándes-Mateo, F.; Dam, T. K.; Brewer, C. F. *Chem. Eur. J*. **2000**, *6*, 1757-1762

the same time, total purification in high yields. After several experiments, utilizing variable amounts of $Pd(PPh_3)_4$, the best conditions found for the coupling were the use of 10% mol of catalyst with respect to compound **1**. Under these conditions, the bis glucoside **3** could be obtained and isolated with a 70% yield.

The stepwise extension of the ethynyl-aromatic chain, was considered the most versatile synthetic approach to obtain increasingly longer molecular wires still decorated with glucosydic pendants. We thus focused on the synthesis of precursor **4** (scheme 4), retaining an aromatic iodo group. From a synthetic point of view **4** is a very interesting system since it maintains a 'free arm' available for a further cross-coupling reaction. It can be differently substituted, for example, with alkynes containing other monosaccharides (e.g. galactose or mannose) or with another kind of naturally occurring derivatives to give asymmetrically substituted systems with potential application in the biological field.

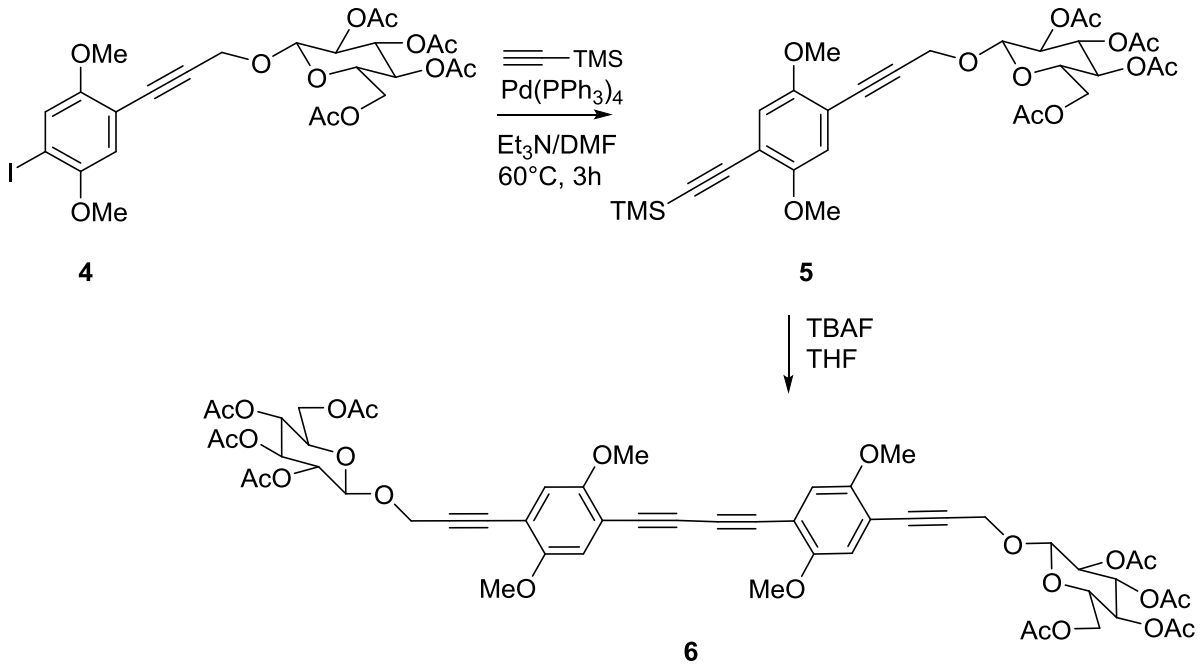
Thus, starting from propargyl glucoside pentaacetate **1** and 1,4-diido-2,5-dimethoxy benzene **2** in a **2**:**1**= 2.1:1 ratio, under the same conditions as above [$Pd(PPh_3)_4$, Et_3N, DMF, 60 ºC] after 3 h, a mixture of the mono-coupled derivative **4** and a small amount of the bis-coupled product **3** was formed from which, the monoioido substituted system 4 could be isolated pure by chromatography in a 70% yield.[6]



Scheme 4

[6] Barattucci, A.; Deni, E.; Bonaccorsi, P.; Ceraolo, M. G.; Papalia, T.; Santoro,A.; Sciortino, M. T.; Puntoriero, F., *J. Org. Chem.* **2014**, *79*, 5113−5120.

With the aim of building up an alternative counterpart exploitable in the Sonogashira reaction, **4** was reacted with a large excess of commercial ethynyltrimethylsilane, giving **5**. The use of a large excess of ethynyltrimethylsilane, allowed an almost quantitative cross-coupling reaction leading to compound 5.



4 **5** **6**

Scheme 5

From a synthetic point of view, compound could be very useful, because it bears a protected alkyne which could be able to react, once desilylated, as alkynyl donor in further Sonogashira reactions. Desilylation of **5** was treated n dry THF solution at -0°C,with a stoichiometric amount of tetra-*n*-butyl ammonium fluoride (TBAF), a typical desilylating agent.[7] Under these conditions, the reaction failed and only the dimeric product **6** was formed. 6 The formation of **6** could be a consequence of the immediate coupling of the reactive deprotected species, initially

[7] Lin, H.-Y.; Huang, W.-C.; Chen, Y.-C.; Chou, H.-H.; Hsu, C.-Y.; Lin, J. T.; Lin, H.-W. *Chem. Commun.* **2012**, *48*, 8913–8915

formed. The reaction was attempted by modulating temperature (-78°), time and dryness of solvents but in any case **6** was the predominant product.

After a bibliographic research to find an alternative route, a modified cross-coupling reaction, adopting Ag_2O as catalytic activator was attempted.[8] This reaction, reported by Farinola *et al*[9] to build up conjugated polymers, was very promising because it allowed the *in situ* formation of a reactive terminal triple bond which could immediately react with an aryl halide present in the reaction medium, under cross-coupling conditions. As suggested by the authors the role attributable to silver oxide could be explained only keeping in mind the simultaneous interaction between silver and iodine and between oxygen and silicon, as shown in figure 1, which would facilitate an almost concerted desilylation and coupling. Both interactions contributed to the proposed transition state from which the coupling product is finally formed in good yield.

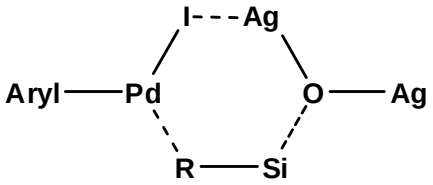
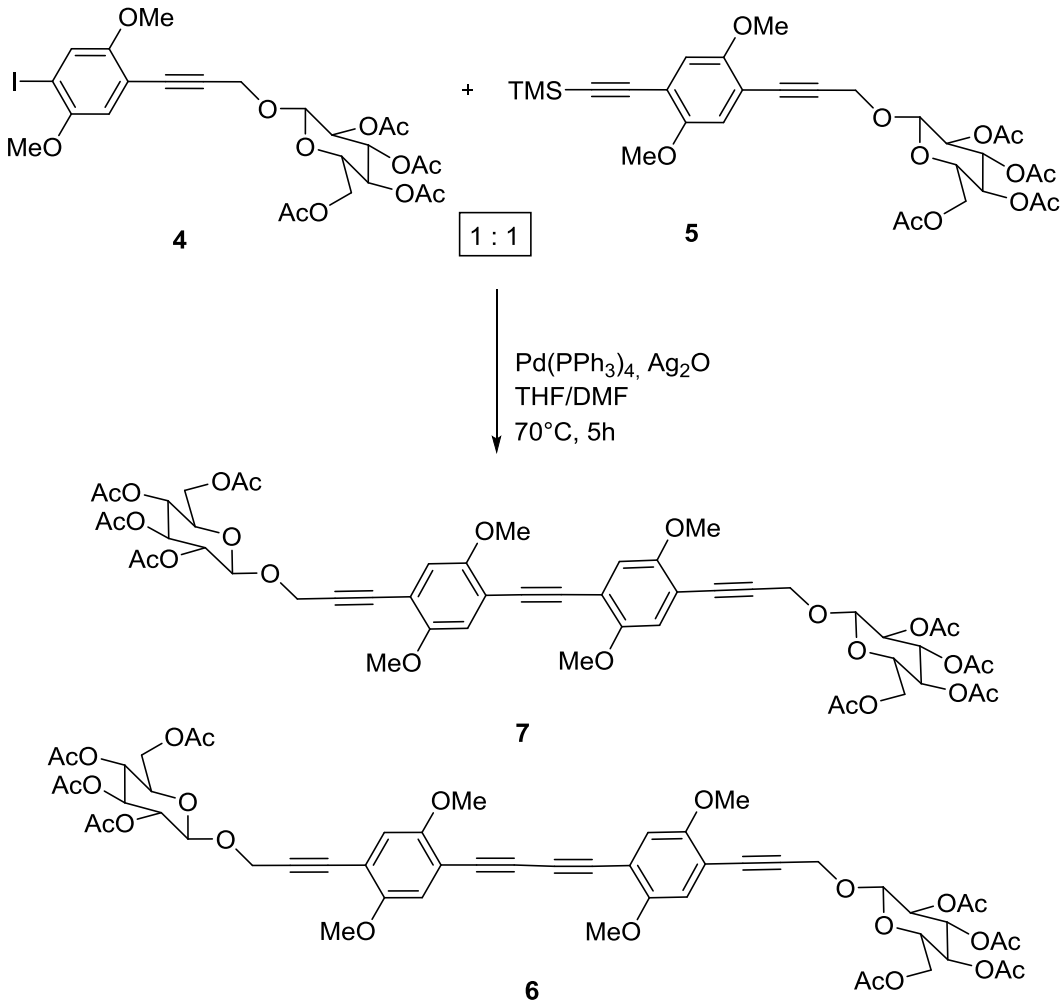


Figure 1

With the aim to build up a new OPE bearing two ethynyl-aromatic unities, the new Ag_2O-modified Sonogashira reaction between an equimolar amount of monoioido derivative **4** and TMS ethynyl aromatic glucoside **5** was attempted (scheme 6), in a mixture DMF/THF (2:1) at 70°C under the same conditions used above. The reaction was complete in 5 hours, but just only after 2 hours a first chromatic change from dark-grey to fluorescent yellow was appreciable The resulting crude reaction was a mixture the expected conjugated luminescent system **7** and a minor amount of compound **6**. , formed by auto-coupling of **5**.

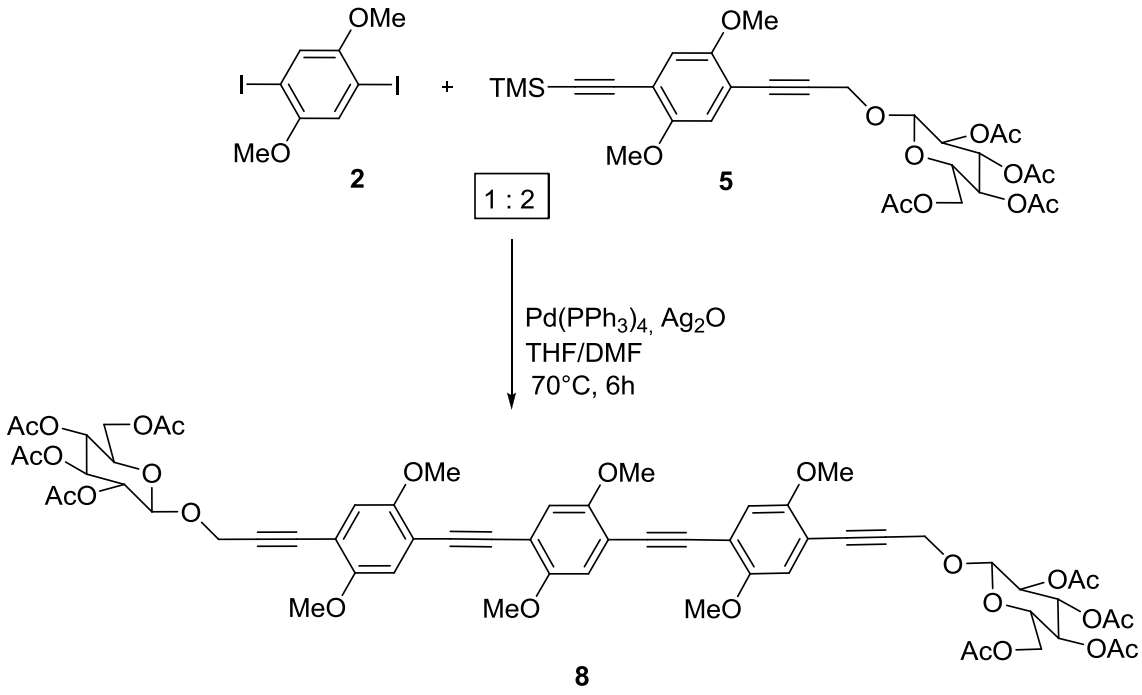
[8] Mori, A.; Kondo, T.; Kato, T.; Nishihara Y. *Chem. Lett.* **2001**, 286-287.

[9] Babudri, F.; Colangiuli, D., Di Lorenzo, P. A.; Farinola, G. M.; Hassan Omar, O.; Naso, F. *Chem. Commun.* **2003**, 130-131.



Scheme 6

In order to further elongate the conjugate chain, we thought of including a new 1,4-dimethoxy substituted aromatic *core* between two monosubstituted glucoderivates. To achieve this goel, 2,5-diiodo-1,4-dimethoxybenzene **2**, was reacted with two equivalents of the monoderivate **5** following the Ag_2O-modified protocol (scheme 7). Under these conditions, compound **8** was obtained with high purity grade after column chromathography, as a glossy yellow crystal in a 64% isolated yield.



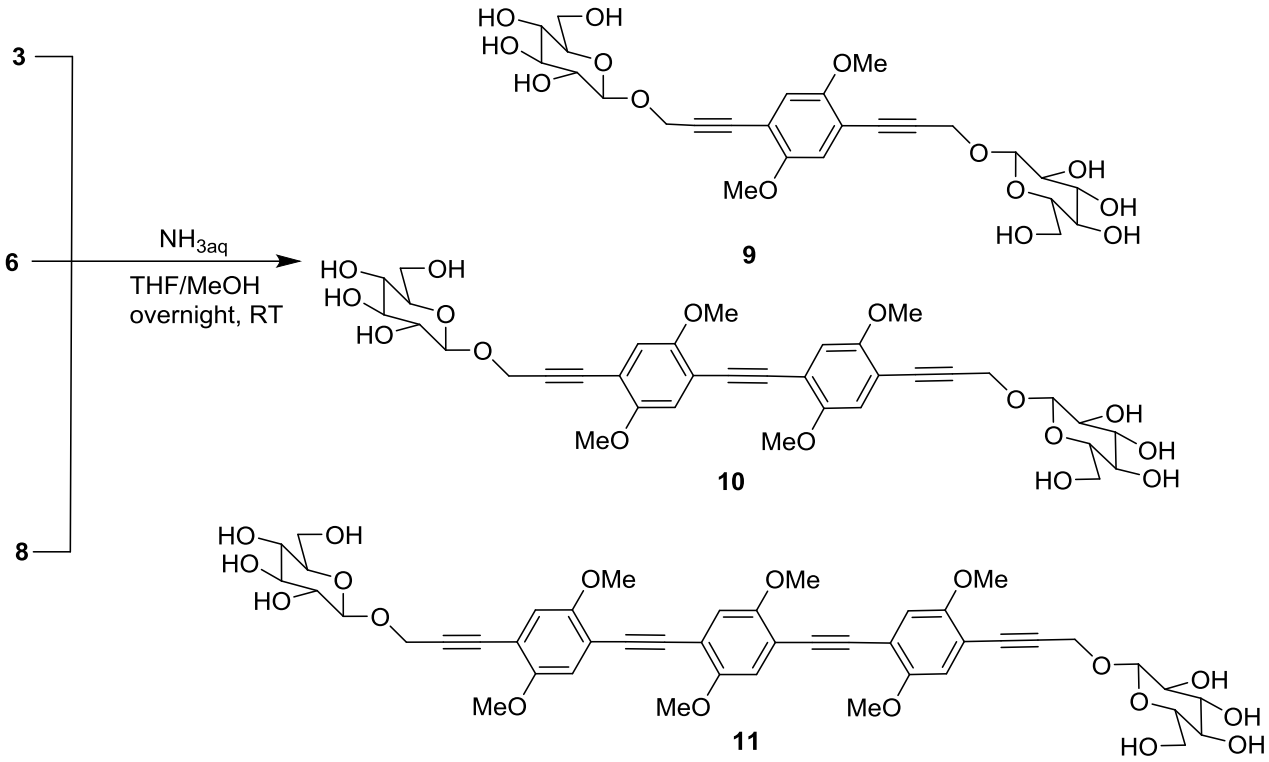
Scheme 7

Once the differently elongated derivatives **3**, **6** and **8** obtained**,** an efficient method to trasform the acetates into free hydroxyl groups was developed. The aim of this synthetic step was the achievement of water-soluble compounds to facilitate the biocompatibility of our systems.

Initially, the deprotection was attempted on derivative **3** following an already known procedure,[10] in the presence of $NH_4OH_{aq.}$ at room temperature and under stirring, in DMF/MeOH overnight. Deacetylated compound **1-OPE-9** was thus obtained with quantitative yield as a crystalline pale-yellow solid. Compounds **6** and **8** were in the same way easily deacetylated, giving quantitavely **2-OPE-10** and **3-OPE-11** respectively.

All the deacetylated products were purified by simple washings of the obtained crude amorphous solids with a mixture of MeOH/Et_2O, to remove the acetamide byproduct.

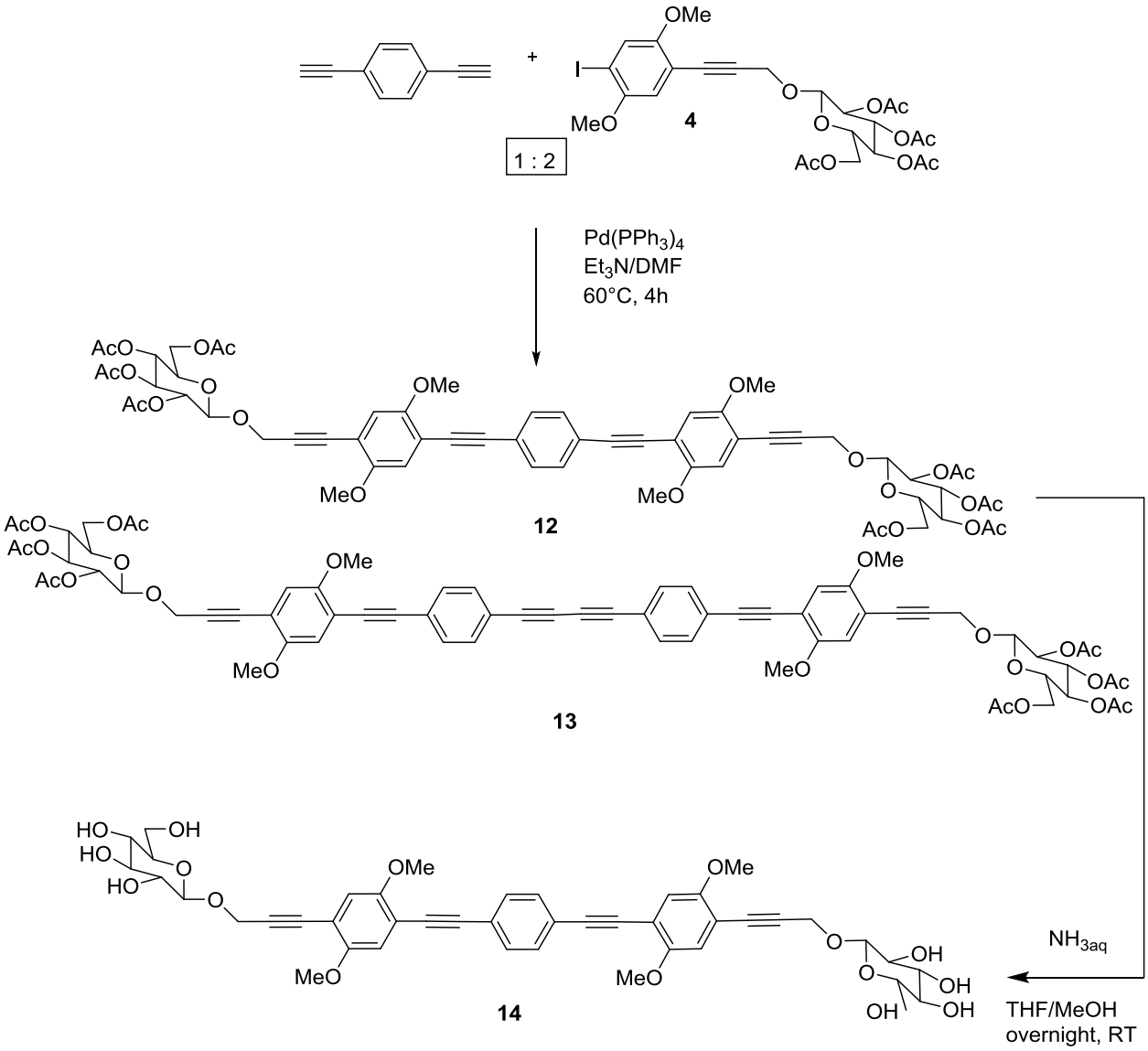
[10] Hasegawa, T.; Numata, M.; Okumura, S.; Kimura, T.; Sakurai, K.; Shinkai, S. *Org. Biomol. Chem.* **2007**, *5*, 2404-2412.



Scheme 8

2.2 Synthesis of OPE glucosides with benzene and N,N-dimethyl aniline cores

With the aim to study which could be the influence of a different aromatic substitution on the photo-physical properties and on the biological behavior of our systems, the synthesis of new oligomers, with a modified central aromatic *core* was envisaged. The convergent synthetic method alloweing the formation of compound **8** (scheme 7) could be applied to a great of variety of new oligomers that could result only by changing the central 'brick' and utilizing the same reaction conditions seen before, in terms of catalysts, solvents, molar ratios and temperature.

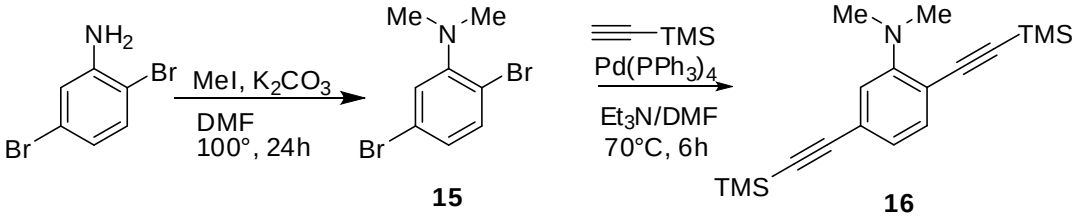


Scheme 9

We firt tried to synthesize the system without substituents on the central aromaticring. With this aim, the monoderivate **4** and commercially available 1,4-diethynylbenzene, wer coupled in 2:1 molar ratio, in the presence of catalytic Pd(0) in a copper-free Sonogashira reaction (scheme 9). The major product of this reaction was the desired compound **12**,, but he crude reaction contained a mixture of **12** and **13** (**12**/**13** 4:1) whose structure corresponded to a double coupling of **4** with two queued central 1,4-diethynylbenzene moieties furnished The chromatographic separation of **12** and **13** was very hard due to their similar *Rf* in the majority of eluant systems.

The only mixture in which they could be separated was toluene/AcCN (95:5) and utilizing a very high weight *ratio* between crude and SiO_2. Once isolated pure, oligomer **12** was deacetylated in the conditions shown in scheme 9 to give free hydroxyl derivative **3-OPE-14** quantitatively as a pale-yellow powder, once the resulting acetamide was eliminated by simple Et_2O washings as mentioned before for **1-OPE-9**, **2-OPE-10**, **3-OPE-11**.

A new system having a central aromatic 'brick' **16**, bearing an electron-donating NMe_2 group, was also efficiently prepared, starting from commercially available 2,4-dibromoaniline (scheme 10). Basic NMe_2 group appeared to be really promising for our purposes: it bears a lone pair on a heteroatom which could cause changes in the absorption and emission spectra of the OPE system and, from a chemical point of view, it has the possibility to be subjected to further and easy functionalization.



Scheme 10

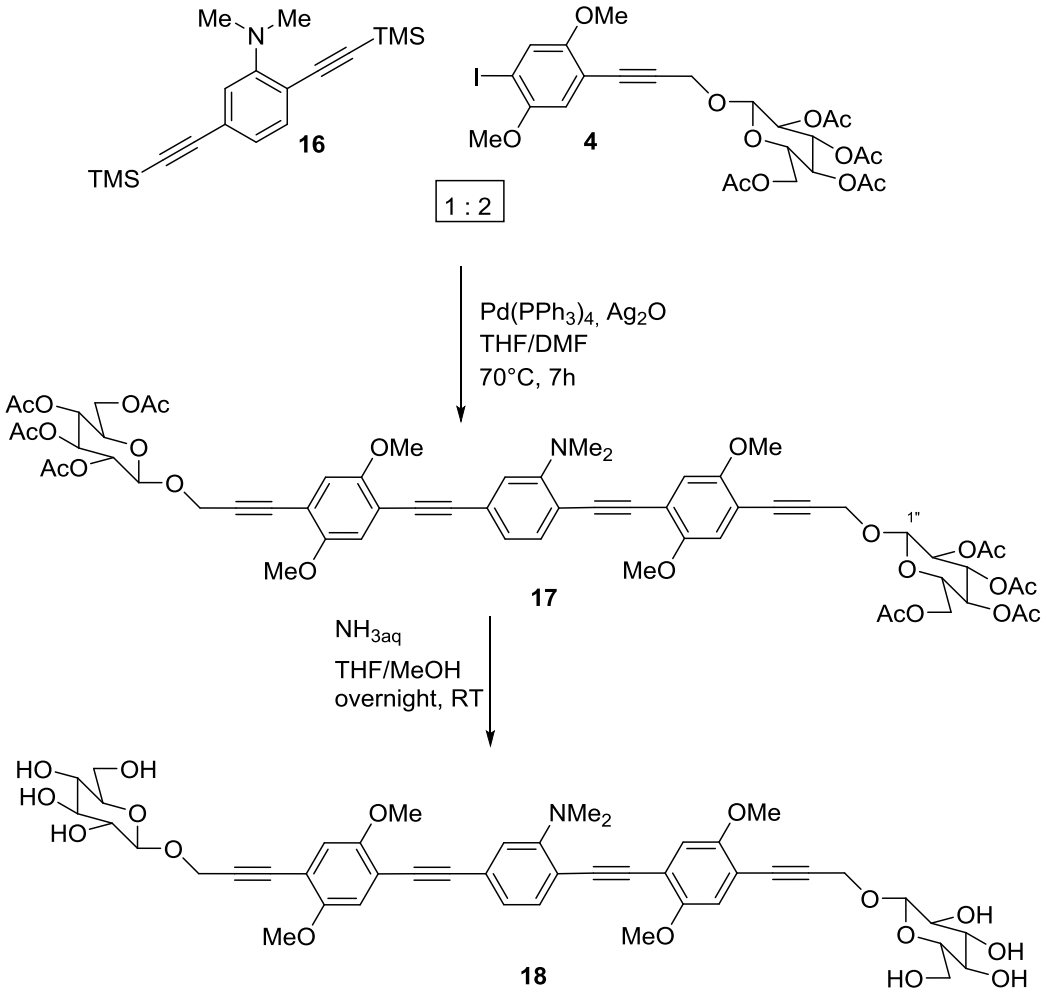
Thus, methylation[11] of the aromatic nitrogen of 2,4-dibromoaniline in DMF with a large excess of MeI and in presence of K_2CO_3 at 100° C, gave dimethyl amino derivative **15** in quantitative yield . Without further purification, compound **15** reacted under copper-free Pd(0) catalyzed Sonogashira coupling[12] conditions, with an excess of ethynyltrimethylsilane (1:12) furnishing **16**

[11] Moroni, M.; Le Moigne, J.; Pham, T. A.; Bigot, J.-Y. *Macromolecules* **1997**, *30*, 1964-1972

[12] Shunichi, O.; Seok, R.C., Heinz, C.; Kung, H. F.*J.Med. Chem.* **2002**, *45*, 4716-4723.

in excellent quantities (Scheme 10). Compound **16** could be separated by a very rapid column chromatography because of the low polarity of the formed compounds.

With compound **16** in hands, the synthesis of OPE **17** was reached with good yields by using the Ag_2O-modified $Pd(PPh_3)_4$ - mediated reaction between bis trimethyl silyl acetylene derivative **16** and **4**. After 7 hours the reaction was complete and resulted in the formation of a highly luminescent compound which could be isolated by chromatography and characterized as **17**.

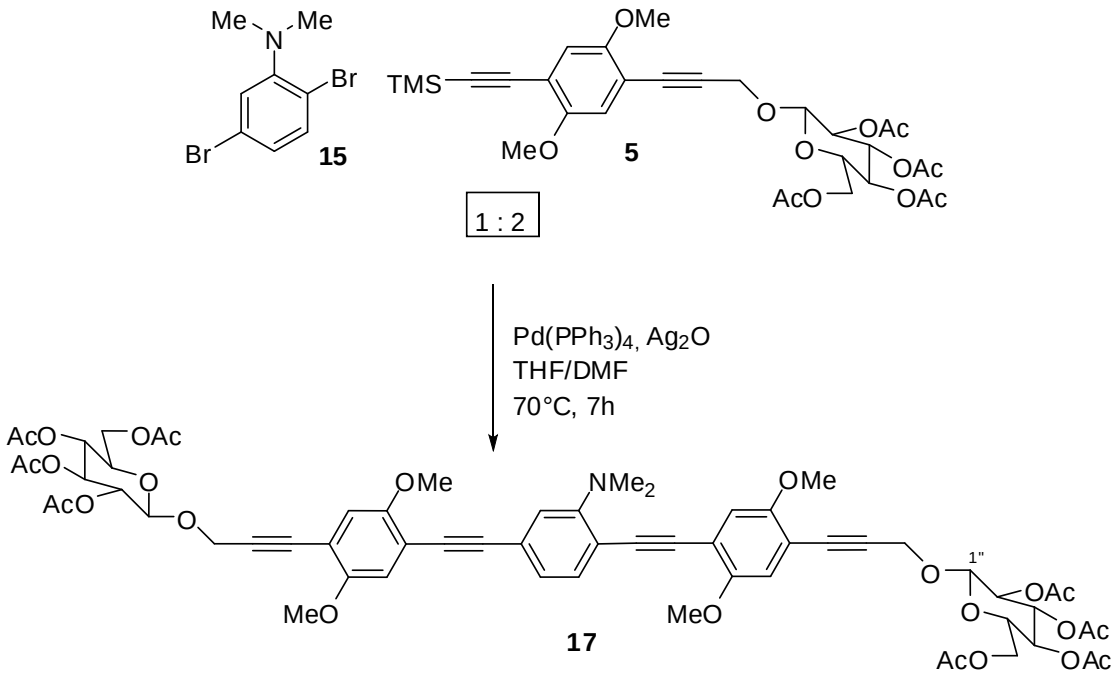


Scheme 11

The protected glucoside **17** was subjected to deacetylation, in the presence of aqueous ammonia. After one night stirring in THF/MeOH at rt, the free carbohydrate derivative **3-OPE-18** was obtained. Further washing of the crude mixture with Et_2O gave **3-OPE-18** as a glossy yellow

crystalline powder in quantitative yield. Compounds **17** and **3-OPE-18** bearing the NMe_2 substituent were stable and easy to handle, in spite of what elsewhere reported[13].

The cross-coupling of **5** with **15** (Scheme 12). was also attempted as an alternative synthetic pathway but, in this case, **17** was obtained in poor yields (<20%), confirming the low reactivity of the dibromoaromatic system involved in cross-coupling reactions.

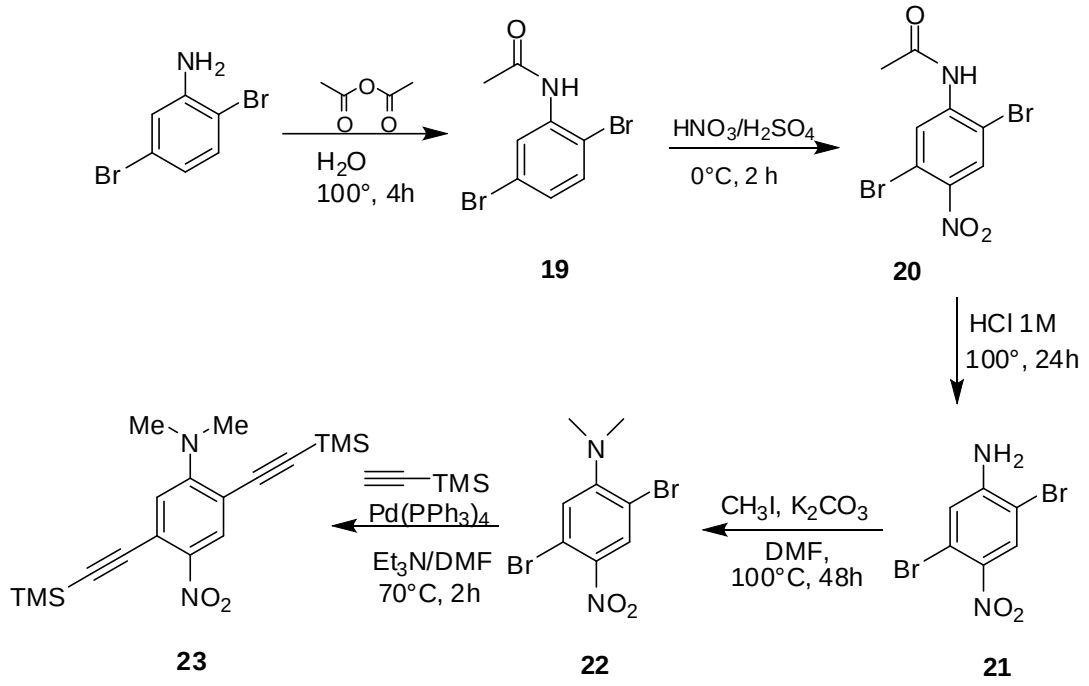


Scheme 12

The insertion of an electron withdrawing group such as NO_2 in *para* position to the dimethylamino one in the central aromatic core of the OPE, following the same synthetic strategy used for the synthesis of **3-OPE-18** (scheme 13) was later tried. To this aim, the synthetic sequence started with the obtention of such central *core* **23** achieved starting from Compound **23**was obtained through the five successive reactions shown in scheme 13. In the first

[13] Yamaguchi, Y.; Tanaka, T.; Kobayashi, S.; Wakamiya, T.; Matsubara, Y.; Yoshida, Z.-i. *J. Am. Chem. Soc.* **2005**, *127*, 9332-9333.

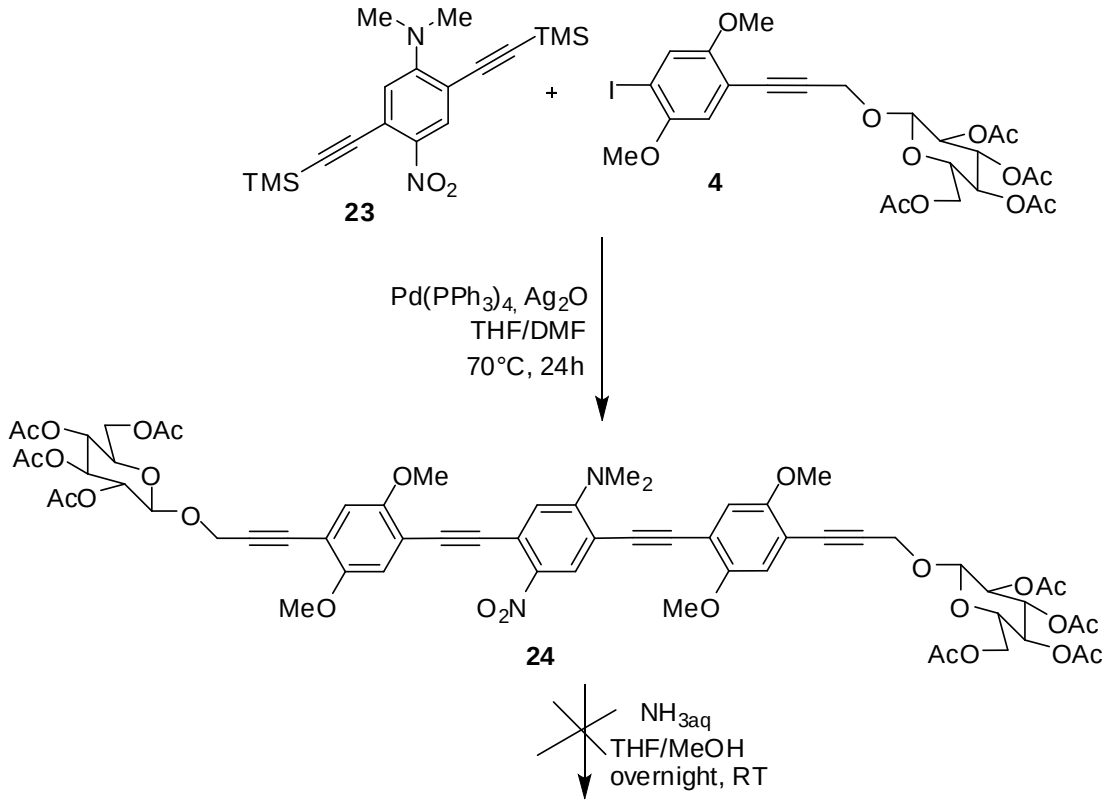
step commercial 2,5-dibromoaniline reacted with acetic anhydride in water, at reflux temperature. After 4 hours the new amide group was formed quantitatively. Without further purification **19** was treated in acid ambient (HNO_3/H_2SO_4), in the typical condition for aromatic nitration, to give compound **20** with good yields (60%). Further deprotetion in acid ambien (HCl 1M) led to the quantitative formation of compound **21**[14].



Scheme 13

Methylation at the aromatic primary amine of **21** followed by cross-coupling of the resulting **22** with an excess of commercially available ethynyltrimethylsilane allowed the access to N,N-dimethyl-4-nitro-2,5-bis[(trimethylsilyl)ethynyl]aniline **23** in good overall yield (70%)

[14] Moroni et al.; *Macromolecules*, **1997**, 30, 1964-1972.

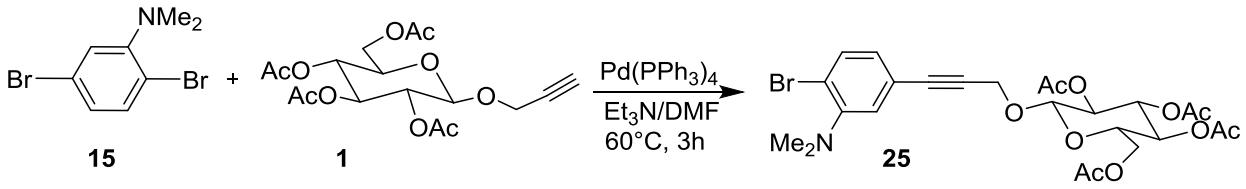


Scheme 14

The synthesis of the coupled product **24** was achieved by the Ag_2O-modified Sonogashira reaction between **23** and **4** under the conditions shown in **Scheme 14**, in 24 hours. Once isolated, **24** was a dark-green oil, highly unstable at room temperature. A test of deacetylation on **24** was attempted, but the reaction gave a very dirty crude mixture impossible to be purified because of its very rapid transformation into decomposition products, even in refrigerated and/or degassed conditions.

Going on with the intention of luminescence modulation of our oligomers, we thought to insert an additional dimethylamino group in the OPE chain. For this scope it was projected to build a new monoderived building block (**25** and its derivatives, scheme 14), analogous to **4** but

containing a dimethylamino substituent at the aromatic ring adjacent to the sugar moiety, able to react in the Sonogashira coupling with different substituted central *cores*.[15]



Scheme 15

Compound **25** was prepared using a Pd(0) cross-coupling reaction from propargyl glucoside **1** and an excess of 2,5-dibromo aniline **15**, as shown in Scheme 14. Under the conditions indicated, compound 25, with only one derivatized arm, was formed in a 75% isolated yield.

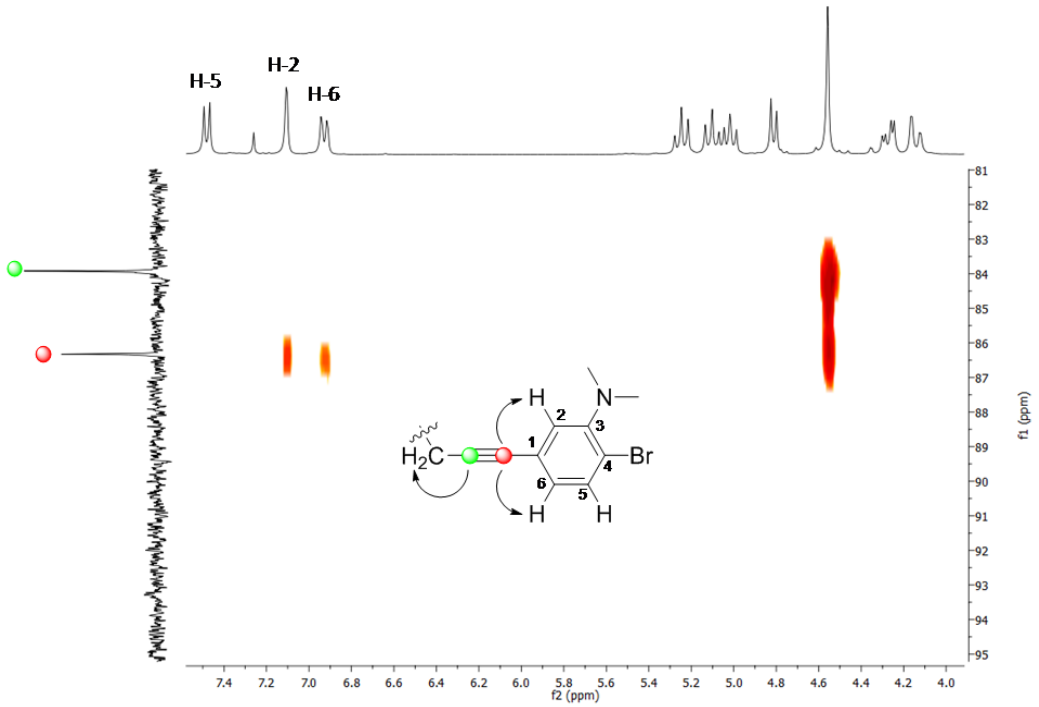
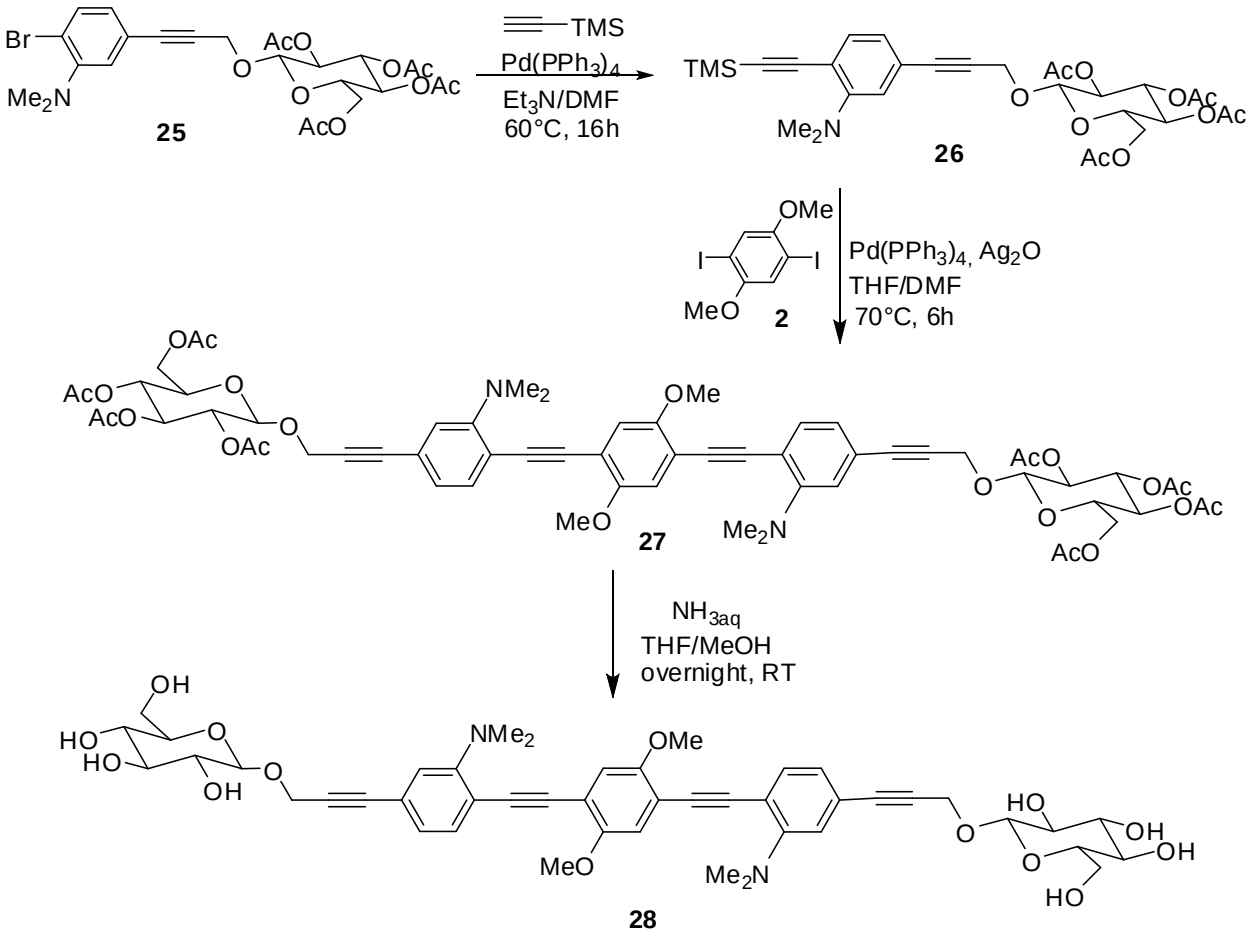


Figure 2

[15] Deni, E.; Zamarrón, A.; Bonaccorsi, P.; Carreño, M.C.; Juarranz, A.; Puntoriero, F.; Sciortino, M.T.; Ribagorda, M.; Barattucci,A., *Eur. J. Med. Chem.*, **2016**, (submitted).

The reaction was completely regioselective and proceeded with the formation of only one product, characterized as the mono susbstituted regioisomer **25**, having the triple bond in *meta* position with respect to to the bulky dimethylamino group, in a 75% yield.

The regiochemistry of compound **25** could be unequivocally demonstrated by the NMR bidimensional experiment HMBC **(**Heteronuclear Multiple Bond Correlation). Significant detail of the obtained spectrum, reporting in ordinate the enlarged ^{13}C region related to the quaternary acetylenic carbons, and in abscissa the aromatic and low-field aliphatic region of the ^{1}H-NMR spectrum, is shown in figure 2. The acetylenic carbon (red) near the aromatic ring shows an evident correlation with aromatic protons H-2 and H-6, allowing to exclude the other possible regioisomer. Moreover, the strong correlation between the other acetylenic carbon atom (green) and the CH_2 groups gives confirmation to the exact structure of compound **25**.



Scheme 16

The cross-coupling reaction of **25** with an excess of commercially available ethynyltrimethylsilane gave rise to the key intermediate **26** in high yield and very mild conditions [$Pd(PPh_3)_4$, Et_3N, DMF, 60 ºC]. The final Ag_2O-modified Pd(0) mediated coupling of 1,4-diiodo-2,5-dimethoxy benzene **2** with **26** (2 equiv.) gave rise to the amino OPE glucoside **27**. Quantitative deacetylation of **27** in the presence of an excess of aqueous ammonia gave the gluco-OPE **3-OPE-28** as a brilliant yellow solid, easily separated from residual acetamide by subsequent washings with MeOH, in almost quantitative yield. All the newly synthesized products are stable and easy to handle.

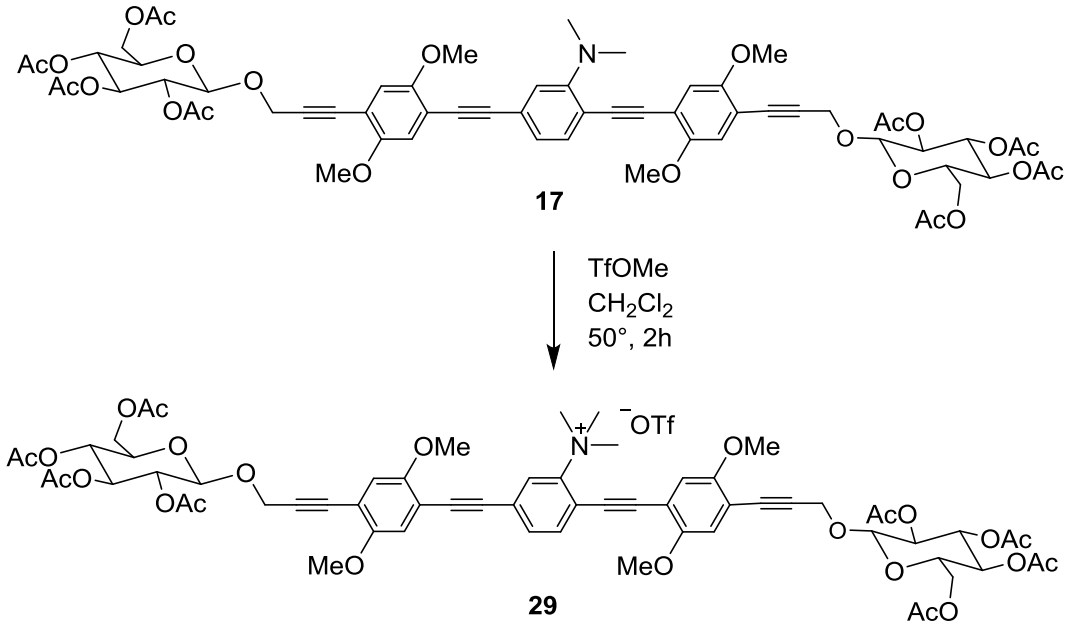
All sugar free compounds **1-OPE-9**, **2-OPE-10**, **3-OPE-11**, **3-OPE-14**, **3-OPE-18** and **3-OPE-28** show, by preliminary tests, low water solubility (up to 10^{-5} M), with the exception of **1-OPE-9**, where the hydrophilic part is predominant and **3-OPE-28**, where it seems that the presence of two dimethylamino groups decreases the hydrophobicity of the conjugated OPEs.

As already illustrated, compounds **17** and **27** possess one or two dimethylamino basic groups consisting in bearing a lone electron pair that, otherwise, can be shared with the conjugated system of the OPEs. With the aim to go on modulating the chemical, physical and biological behavior of our conjugated glucosides, it was thought to transform the $–NMe_2$ groups into quaternary ammonium salts $–NMe_3^+$. In fact, the presence of a charged group could increase the solubility of the synthesized molecules in water and at the same time the absence of the lone pair on nitrogen could cause an alteration of the photo-physical properties.

The first test of quaternization was performed using the typical methylating agent, iodomethane. Reaction of **17** with a large excess of iodomethane in refluxing acetone at didn't lead to the formation of the expected quaternary product. By changing solvent, in order to increase the reflux temperature, **17** was tried to react with CH_3I in acetonitrile, but even after 12 hours in these conditions no reaction occurred.

The lack of reactivity of **17** is attributable to the low basicity of the aromatic nitrogen. For this reason it was necessary to use a stronger methylating agent like methyl

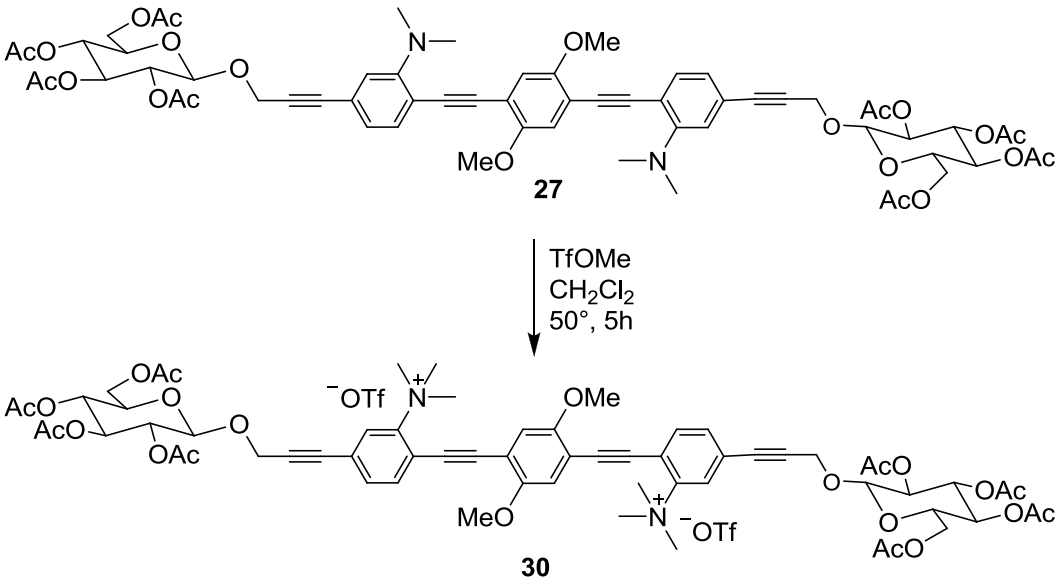
trifluoromethanesulfonate (TfOMe). TfOMe was slowly added to a solution of **17** in CH_2Cl_2 in 1:1 molecular ratio, and the solution was heated up to the reflux temperature for 2 hours leading to the quantitative formation of the quaternized product **29**. It was observed that a strict molecular ratio between **17** and triflate has to be maintained because a larger amount of TfOMe, which contains a small but critical amount of triflic acid, caused the decomposition of **29**. Purification was not necessary because, after evaporation under *vacuo* and following washings of the obtained solid with Et_2O, the product **29** was obtained as an orange crystalline pure powder.



Scheme 17

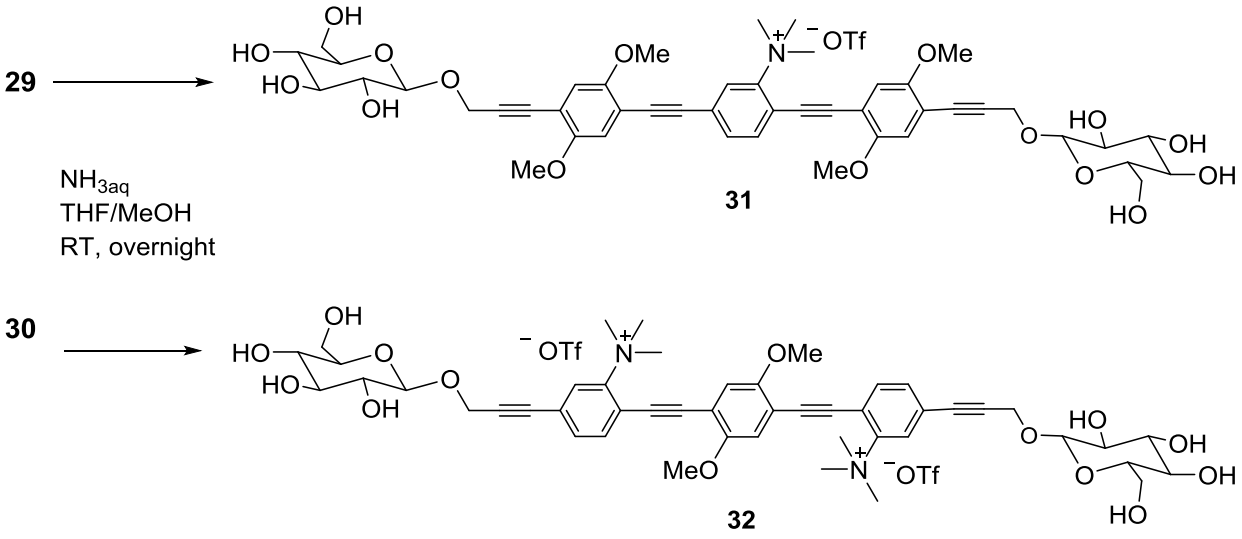
A good success in terms of yield of the above described reaction is to utilize of properly stored methyl trifluoromethanesulfonate (freezed and under inert atmosphere) because it is extremely sensitive to air and heat.

The same reaction was performed on the OPE **27**, having two dimethyl amino groups, with the aim of obtaining the doubly quaternarized salt. Thus, slow addition of TfOMe to a solution CH_2Cl_2 of **27** in **27**/TfOMe: a 1/2 molecular ratio, afforded the double quaternary ammonium salt **3-OPE-28** in quantitative yield as a brilliant orange powder.



Scheme 18

Deacetylation of **29** and **30** was performed in the presence of large excess of NH_{3aq} in MeOH and THF giving **31** and **32** respectively (scheme 19).



Scheme 19

2.3 Photo-physical measurements and singlet oxygen evaluation

In order to determine how the elongation of the conjugated system and the presence of different substituents on the aromatic rings could modulate the photo-physical properties of the synthesized OPEs, the photo-physical studies were performed on all the obtained compounds. This part of the PhD thesis was developed in collaboration with Prof. FaustoPuntoriero, from the University of Messina.

To gain insightinto thelight-activatedactivity of the oligomers, we carried out systematic photophysical studies, both in organic solvents and in acqueous buffered solution.

These studies have been performed not only for the strictly characterization of the excited state of OPEs but also to investigate their ability as photosensitizers for singlet oxygen generation.

QUI devispiegarequaiproprietà si vanno a misurare (UV e amissione di luminescenza) e che informazioni ti daranno. Dovrestispiegare brevemente ildiagramma di Jablonski per giustificare la formazione di ossigenosingolettoìespiegare come si calcola la resaquantica. Questo è moltio importante.

Reactions of singletoxygen$^1O^2$*($^1\Delta$g), are of much current interest because of their importance in many photo oxidations of chemical and biological systems including reactions used in photo-chemotherapy. There is strong evidence for the involvement of singlet oxygen, a powerful oxidant, in many photosensitized oxidations, photodynamic inactivation of viruses and cells, in phototherapy for cancer, in photo carcinogenisis, in the photodegradation of dyes and polymers and in the dyes ensitization of the photodegradation of polymers.

In solution, the singlet oxygen is often prepared by a process called photosensitization. Photosensitized generation is a simple and controllable method for the production of singlet oxygen, requiring only light of an appropriate wavelength, and a photosensitizer capable of absorbing and using that energy to excite oxygen to its singlet state.

A photosensitizer is irradiated to its singlet excited state, followed by intersystem crossing to its triplet excited state.

Intersystem crossing generates the sensitizer triplet state,T1. The lifetime of the T1 state is longer (ms) than that of the S1 state (ns) allowing this excited state to react in one of two ways, defined as Types I (radical reactions) or Type II process (singlet oxygen production), see Figure 3.[16]

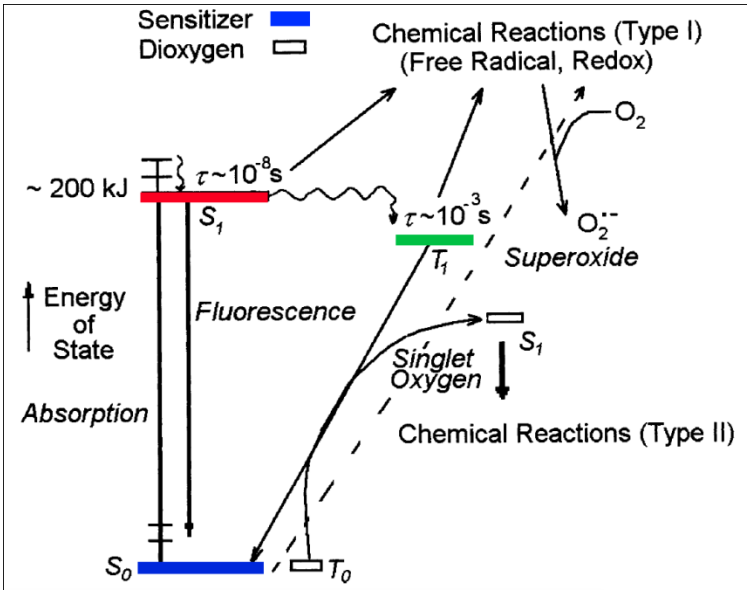


Figure 3. Generation of excited photosensitizer states and reactive dioxygen species.

All the photophysical experiments were performed at room temperature using as solvents both CH_2Cl_2andphosphate buffer (100mM PBS, pH=7.0).

All spectroscopic and photophysical data are reported in table 1: for the absorption, the maxima (or shoulders) of the lower energy bands are given. Some direct comparison of absorption/emission spectra in DCM are given in figures 4, 5 ad 6.

[16] R. Bonnett, Chem. Soc. Rev. (1995) 19.

compound	Absorption λ max, nm (ε/ $M^{-1}cm^{-1}$)		Luminescence, 298 K					
			λ_{max}/ nm		ϕ		τ/ns	
	DCM	PBS	DCM	PBS	DCM	PBS	DCM	PBS
3/1-OPE-9	344 (11000)	344 (11400)	383	384	0.45	0.46	0.72	0.75
7/2-OPE-10	378 (35000)	379 (34800)	404	404	0.87	0.89	1.54	1.60
8/11	390 (49500)	390 (50000)	433	433	0.85	0.83	0.98	0.96
12/3-OPE-14	377 (39500)	378 (39000)	437	437	0.82	0.80	0.76	0.74
17/3-OPE-18	388 (38900)	389 (39300)	472	473	0.57	0.58	2.47	2.40
24	381 (38000)	380 (38500)	-	-	-	-	-	-
27/3-OPE-28	392 (40000)	391 (40500)	474	475	0.74	0.76	3.54	3.50
29/31	400 (41900)	400 (41900)	470	471	0.90	0.90	2.10	2.00

Table 1. Absorption and emission data at room temperaturein dichloromethane (DCM) and phosphate buffer (BPS) solution.[a]

a) Dichloromethane have been used for the protected species (**3**, **7**, **8**, **12**, **17**, **24**, **27**, **29**) whereas PBS for the deprotected ones (**1-OPE-9**, **2-OPE-10**, **3-OPE-11**, **3-OPE-14**, **3-OPE-18**, **3-OPE-28**, **31**, **32**).

The absorption spectra of all the investigated species, in DCM solution (for the protected **3**, **7**, **8**, **12**, **17**, **24**, **27**, **29**) as well as in aqueous solution (buffer phosphate for the deprotected **1-OPE-9**, **2-OPE-10**, **3-OPE-11**, **3-OPE-14**, **3-OPE-18**, **3-OPE-28**, **31**, **32**), are characterized by an intense absorption in the UV region of λ=340-400 nm (ε in the 10^4-10^5 M^{-1} cm^{-1} range), due to spin-allowed π–π∗ transitions of the aromatic moieties. A batochromic effect is observed by increasing the number of ethynylene aromatic moieties, as a consequence of the increased conjugation. Most oligo(phenylene-ethynylene)sform aggregates and excimers when added to solvent/nonsolvent (solvent is able to dissolve the species whereas a non solvent is not) mixtures,

which are usually detected by absorption and fluorescence spectroscopies. In our case the concentration used was low enough to avoid these feature: concentration dependence was observed only when higher than 10^{-4} M and in mixed solvents.[17]

With respect to the emission spectra, it is possible to observe that by increasing of the chain length, moving from **3** to **8** (passing from 1 to 3 phenylenethynylen unities) the emission energy decreases whereas the emission quantum yield doubles. This is reminiscent of the bathochromic shift in the corresponding absorption spectra.

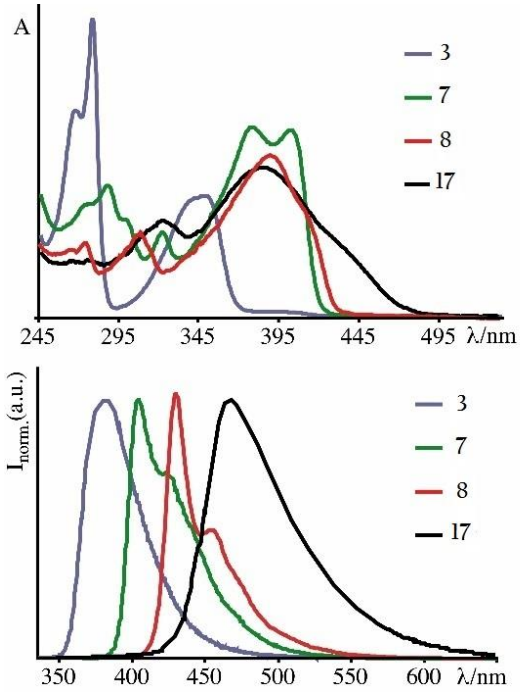


Figure 4. Absorption (top panel) and emission spectra(bottom panel) of **3**, **7**, **8** and **17.** Please note that the absorption spectra are not reported in epsilon to better distinguish the absorption profile each other (for the epsilon value please refer to Table 1).

The augmented π conjugation between the peripheral subunits in **8** and **12** causes a further decrease of both luminescence excited state energy and quantum yield. By increasing the chain lengtht red shift of the absorption maximum is registered (see Table 1, Figure 4): this behaviour

[17]Babudri, F.;Colangiuli, D.; Di Bari, L.; Farinola, G. M.; Hassan Omar, O.,Naso, F.; Pescitelli, G. *Macromolecules***2006**, *39*, 5206-5212.

is consistent with a greater π-conjugation passing from **3** to **8**/**12**. The molar extinction coefficients (ε) of the lowest-energy transition also increase with the same trend as it is possible to observe in Table 1.

The insertion of one electron-donating group (NMe_2) on the central subunit of the oligomer **17** shifts the luminescence to the red, while the quantum yield decreases, still remaining at the high value of 0.57. The unstructured large emission profile suggests that in this case the excited state has a partial charge transfer character that could be tentatively attributed to n→π* transitions(see Table 1, Figure 4). The absence of luminescence in **24** can be adscribed to the presence of the NO_2 electron-withdrawing group that acts as a quencher for these systems.[18]It is important to stress that no changes in the photophysical properties havebeen observed between the acetylated compounds and the corresponding deacetylated ones, in DCM and aqueous solution.

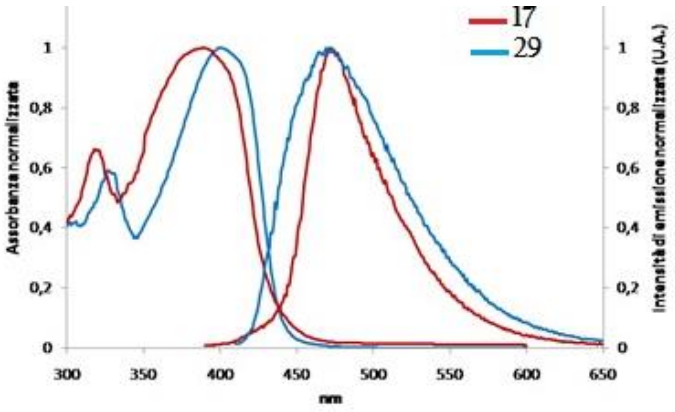


Figure 5. Normalized absorption and emission spectra of **17** (red line) and **29** (blue line in DCM solution.

A direct comparison between the absorption and emission spectra of compound **17** and its corresponding quaternary ammonium salt **29** is shown in Figure 5.

[18]Yamaguchi, Y.; Tanaka, T.; Kobayashi, S.; Wakamiya, T.; Matsubara, Y.; Yoshida, Z.-i. *J. Am. Chem. Soc.* **2005**, *127*, 9332-9333

The absence of the lone pair on nitrogen in **29**, due to the quaternization of dimethylamino group, provoked a further shift to red in the absorption, probably due to the fact that the electron-donating ability of amines is inverted upon protonation or quaternization. An interesting observation correspond to the significant increase of the quantum yield of emission of **29** compared to **17** as a consequence of the loss of the lone pair. In fact the lone pair can act as electron donor to the excited state in **17**, activating a further non radiative deactivation pathways via partial population of a charge separated state. The absence of this deactivation channel in **29** brings to an higher quantum yield and longer emission lifetime.

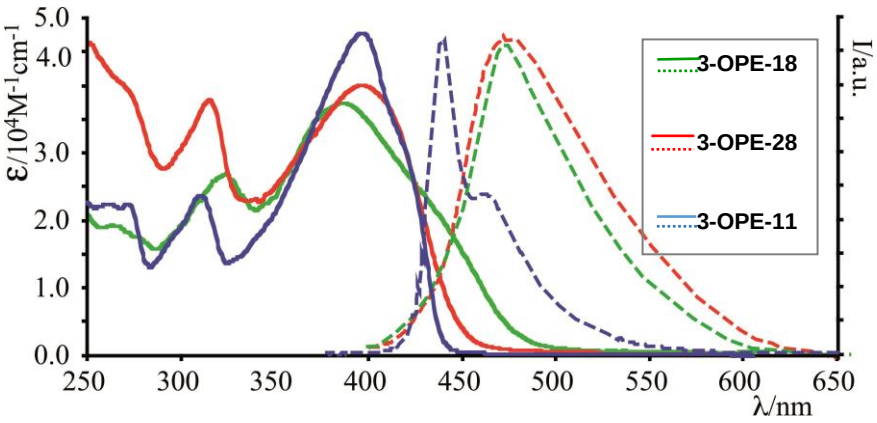


Figure 6. Absorption and emission (dashed lines) spectra of hydroxyl derivatives **3-OPE-11**, **3-OPE-18** and **3-OPE-28**

Figure 6 shows a comparison of absorption and emission spectra of three selected deacetylated trimers: **3-OPE-18**, **3-OPE-28** and **3-OPE-11**. The absorption spectra of the OPEs **3-OPE-18** and **3-OPE-28**, in aqueous solution (buffer phosphate) are characterized by intense broad low-energy absorption bands centered at λ=388 nm and at 392 nm, respectively with ε values in the range 10^4-10^5 M^{-1} cm^{-1}. Theseabsorptions in the UV region are due to spin-allowed π–π* transitions centred on the aromatic skeleton of OPEs[19]. Excitation of **3-OPE-18** and **3-OPE-28**

[19]Lanoë, P.-H.; Gallavardin, T.; Dupin, A.; Maury, O.; Baldeck, P. L.; Lindgren, M.; Monnereau, C.; Andraud, C. *Org. Biomol. Chem.***2012**, *10*, 6275-6278.

in the range 280–420 nm allowed the appearance of emissive state bands in the blue region of spectrum. Interestingly, a closer look at the results presented in Table 1, summarized below (Figure 6) revealed clear differences between the photophysical properties of **3-OPE-18** and.In particular, moving from **3-OPE-18** to **3-OPE-28**, the emission quantum yield increased [from Φ= 0.57 (**3-OPE-18**) to Φ=0. 74 (**3-OPE-28**)] as well as the excited state lifetime, measured by "Time correlated single photon counting" technicals [from τ/ns = 2.47 (**3-OPE-18**) to τ/ns 3.54 (**3-OPE-28**)].

Luminescence quantum yields (ϕ) were determined by measuring luminescence spectra of an air-equilibrated ethanol solution of anthracene (Φ= 0.2)[20] and luminescence spectra of OPEs in air equilibrated solution using identical acquisition parameters. In equation 1, *I* is the integrated luminescence intensity, *A* is the absorbance at the excitation wavelength, and *n* is the refractive index of the solvent. The subscript "*r*" refers to a reference substance with known luminescence quantum yield (ϕ_r)

$$\phi = \phi_r \cdot \frac{I \cdot A_r \cdot n}{I_r \cdot A \cdot n_r}$$

Equation 1

Interestingly, both quantum yields were reduced with respect to compound **3-OPE-11** (Φ=0.85) bearing two methoxy groups as substituents at each aromatic ring, and in which the dimethylamino group is not present. This behaviour could suggest that the excited singlet state decays faster in **3-OPE-18** because, in this case, the rate of intersystem crossing to the triplet excited state (responsible for sensing singlet oxygen) is enhanced with respect to **3-OPE-28**.

[20]a) Demas, J. N.; Crosby, G. A.J. Phys. Chem.197,75, 991−1024. (b) Dempster, D. N.; Morrow, T.; Quinn, M. F.J. Photochem.1974,2, 329−341

Considering that **17** and **3-OPE-28** have the longer lifetimes, probably due to a partial intersystem crossing character of the radiative deactivation, we thought to examine in depth their photo physical properties, in particular to determine the production of singlet oxygen ($^1O_2$), which is the key species for further biological evaluation.

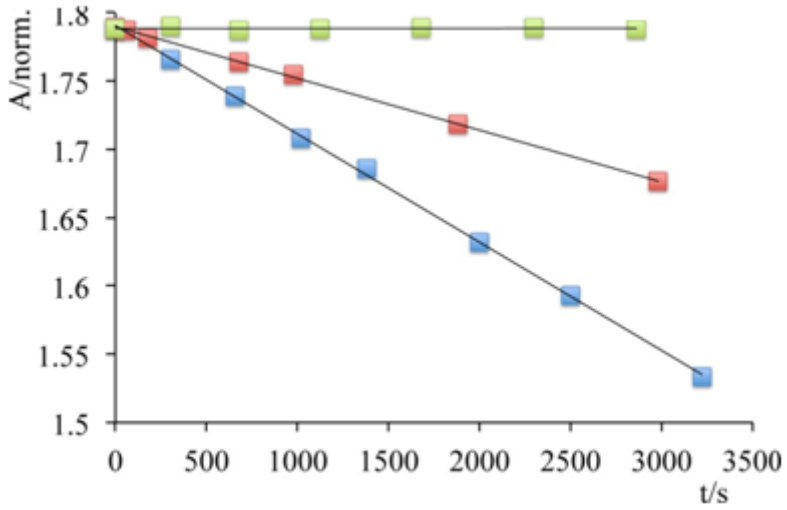


Figure 7: Degradation of uric acid (λ= 292 nm) versus Time using as sensitizer **3-OPE-18** (blue squares), **3-OPE-28** (red squares) and the model compound **3-OPE-11** (green squares). The OPEs solution concentrations are $5x10^{-5}$M.

On the basis of this assumption, we performed an indirect evaluation of singlet oxygen production by excitation of the oligomers **3-OPE-18**, **3-OPE-28** and **3-OPE-11**. The singlet-oxygen generation was evaluated by a chemical method using uric acid (UA) as detector.[21]Under irradiation at neutral pH, uric acid is stable and shows one absorption band centered at 292 nm. When singlet oxygen (or ROS) is photo-produced, uric acid is irreversibly oxidized as highlighted by the reduced intensity of its absorption band. We used the methylene blue species

[21]a)Passarella Gerola, A.; Semensato, J.; Silva Pellosi, D.; Roberto Batistela, V.; Ribeiro Rabello, B.; Hioka, N.; Caetano, W., *J. Photochem. Photobiol. A* **2012**, 232, 14-21. (b) Ribeiro Rabello, B.; Passarella Gerola, A.; Silva Pellosi, D. ;Tessaro, A. L. ; Aparício, J. L.; Caetano, W.; Hioka, N., *J. Photochem. Photobiol. A.* **2012**, *238*, 53-62.

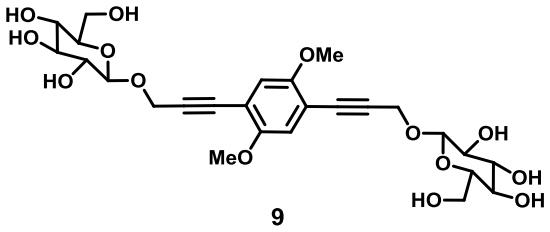
as a reference photosensitizer. The decay curves of the UA absorption band at 292 nm as a function of the irradiation time in the presence of **3-OPE-18** (blue squares), **3-OPE-28** (red squares) and **3-OPE-11** (green squares) are reported in Figure 7 (concentrations $5x10^{-5}$M). The singlet oxygen quantum yields obtained for **3-OPE-18** (Φ_{1O2} 0.15) and **3-OPE-28** (Φ_{1O2} 0.09), listed in Table 1, are in line with the photophysical results above discussed, confirming that the reduced luminescence quantum yield for **3-OPE-11** could be ascribed to a more efficient sensitization of the triplet excited state of these species.

2.3 Biological studies

2.4.1. Cell internalization

Once studied the photo physical properties, we thought to investigate the ability of these new oligomers to cell internalization. This first part of the biological study was undertaken in collaboration with Prof. Maria Teresa Sciortino, from Messina University.

As a first step we performed uptake experiments for compounds **1-OPE-9**, **2-OPE-10**, **3-OPE-11**, **3-OPE-18** and **3-OPE-28** on HEp-2 cells (cells from epidermoid carcinoma larynx tissue). Compounds were dissolved in DMSO in order to have a final concentration of 10mM. HEp-2 cells were grown on culture slides and treated with the products to a final concentration of μM (in RPMI medium) for 24h. The internalization in the cells was observed with fluorescence electronic microscopy using blue filter (DAPI) or green filter (FITCH).



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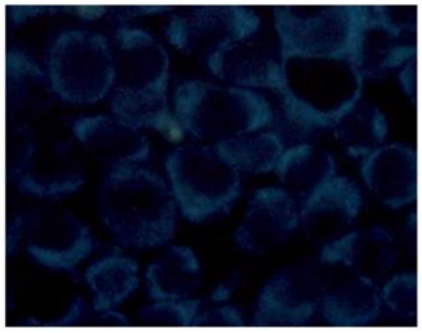
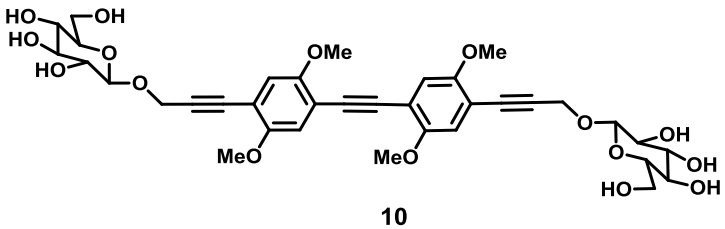


Figure 8

The shortest glucoside **1-OPE-9** incubated for 24 h slightly internalized in HEp-2 cell lines (figure 8), the internalization is facilitated by the presence of the glucosidic substituents deacetylated.

Glucoside **2-OPE-10** was unable to internalize probably as a consequence of the elongation of the aromatic chain which renders the structure more lipophilic, but it was blocked on the cellular membrane as ordered and fluorescent sticks. In figure 1-OPE-9 it is possible to observe the fluorescence with blue filter (DAPI) and with green filter (FITCH).



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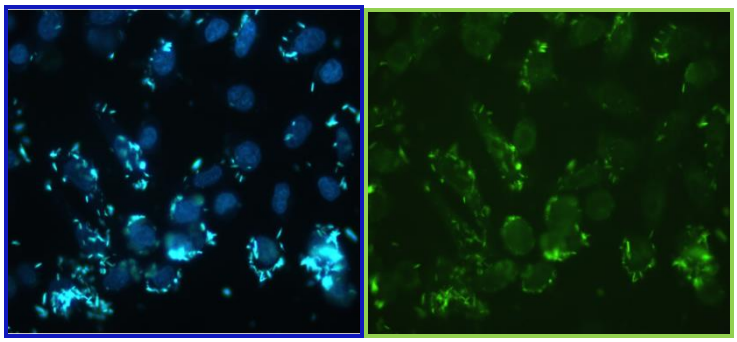
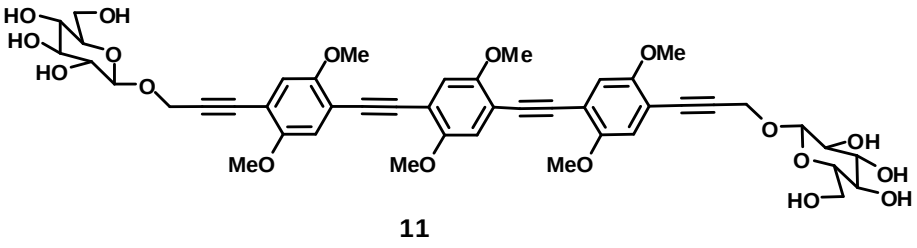


Figure 9

As expected, compound **3-OPE-11** was completely unable to be uptaken in Hep-2 cells due to its elongated ethynyl-aromatic chain.



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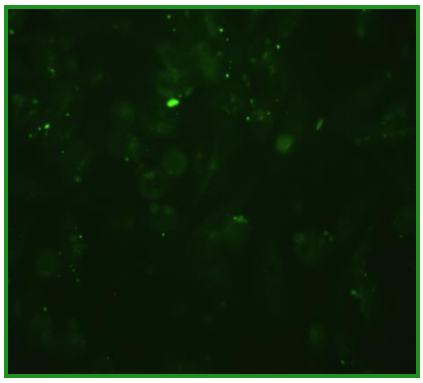


Figure 10

On the other hand, only changing the aromatic *core* substitution, and then passing from **3-OPE-11** to **3-OPE-18**, with a dimethyl amino substituted aromatic core, massive internalization happens. Figure 10 refers to the cell internalization and efficient enlightening of glucoside **3-OPE-18**, showing the main localization in vesicles within the cytoplasmic compartment. Particularly, it is represented the fluorescence microscopy images of HEp-2 cells incubated with

3-OPE-18 (100 μM) and analyzed using a FITC filter (green emission) or a DAPI (blue emission). The more lipophilic OPE glucoside **17**, acetylated analogue of **3-OPE-18**, lightly internalized Hep-2 cells.

After these observations and experimental results it is possible to conclude that the massive internalization of **3-OPE-18** is due to the synergic effect of several contributions: i) elongation of the hydrophobic chain, going from glucosides **1-OPE-9** to **3-OPE-11**; ii) substitution of the aromatic central core with a dimethylamino group in **3-OPE-18** instead of a bis-methoxy moiety present in glucoside **3-OPE-11**; iii) deacetylation of the sugar moieties, improving the hydrophilicity of **3-OPE-18** with respect to glucoside **17**.

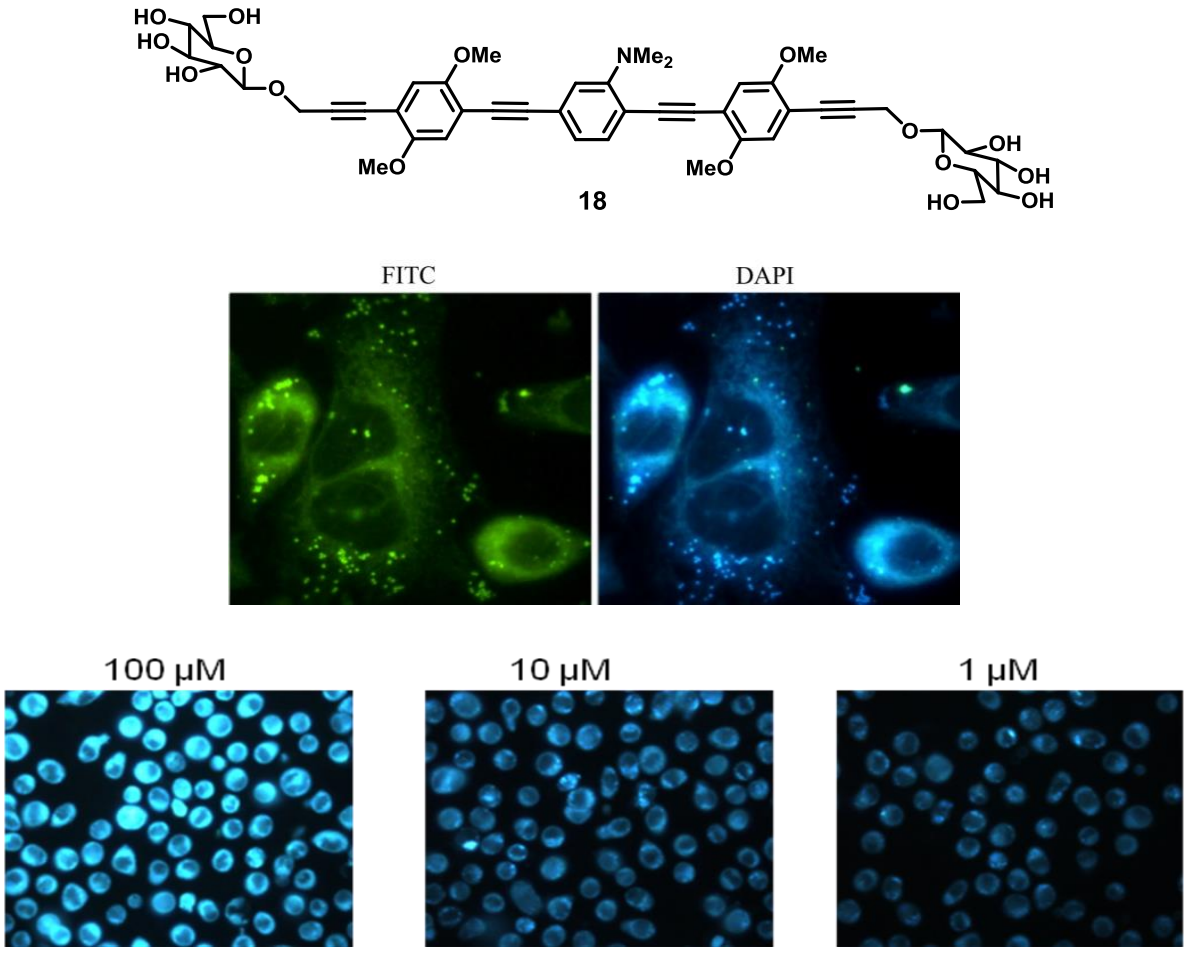
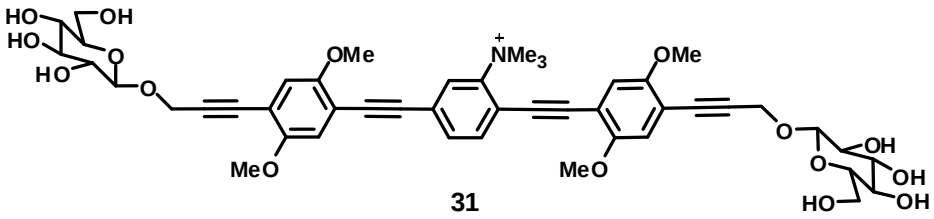


Figure 11

The great luminescence quantum yield and the high degree of endocytosis of **3-OPE-18** permits to detect fluorescence emission even when cells are incubated at low concentrations up to 1µM (Figure 11).

As last, the cell internalization of OPE compound **31**, having a quaternary ammonium salt. in HEp-2 cells was evaluated by fluorescence microscopy with DAPI filter (Figure 12).

The high fluorescence of compound **31** provoked a blurry image but it is plausible to affirm that **31** internalized and that it was situated on the nuclear cellular membrane.



31

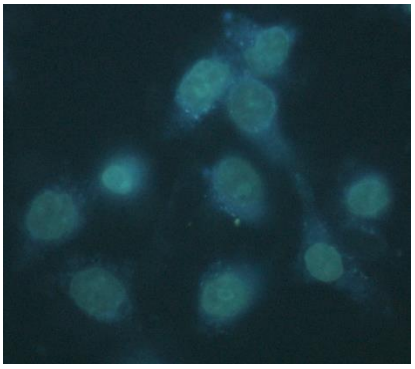


Figure 12

2.4.2. OPEs photosensitizers in PDT.

Having in our hands the first results in cell internalization of **3-OPE-18** and photophysical study of **3-OPE-18** and **3-OPE-28**, including excited state lifetimes and singlet oxygen productions (2.2), we thought to study the possible application of **3-OPE-18** and **3-OPE-28** as photosensitizers (PSs) in photodynamic therapy (PDT). This part of the work was carried out in collaboration with Prof. Ángeles Juarranz Torres from the Universidad Autónoma de Madrid.

Phototherapy is a minimally invasive therapeutic procedure already approved for the treatment of various oncological and non-oncological pathologies of skin. Variants of phototherapy include targeted ultraviolet B (UVB) phototherapy, topical psoralen plus ultraviolet A (PUVA), and photodynamic therapy (PDT). All of them are localized forms of phototherapy to treat skin lesions, either as first-line treatment or as complementary treatment for those that do not respond to other topical treatments.[22]

PDT are based on the specific accumulation of a photosensitizer (PS) in the target tissue, followed by irradiation with light at a wavelength matching the absorption spectrum of the photosensitizer. Upon light absorption, the PS transits from its ground state to an unstable excited singlet state, from which it can decay, either to the ground-state or to the excited triplet state. In this long-lived excited triplet state, the PS is able to produce singlet oxygen ($^1O_2$) which in turn gives rise to other reactive oxygen species (ROS), such as peroxides, superoxide anions or hydroxyl radicals. These species are able to react directly with a biological substrate. ROS can oxidize vital cellular components inducing an acute cell stress response culminating in cellular death, mainly by apoptosis and/or necrosis. The cell death pathway depended, among other factors, on the nature and intracellular localization of the PS and on tumor properties.

The ideal PS should possess high photo-activity and selective photocytotoxicity for sick skin cells but low dark toxicity, activation at discrete wavelenghts, efficient and fast distribution and elimination from tissues and chemical and physical stability. The amphiphilic nature of PSs, having lipophilic and hydrophilic moieties, is also an important feature for their application in biological tests. Although a significant number of photosensitizers are synthesized each year,

[22] (a) Almutawa, F.; Thalib, L.; Hekman, D.; Sun, Q.; Hamzavi, I.; Lim, H.W., *Photodermatol. Photoimmunol. Photomed.* **2015**, 31, 5-14. (b) Situm, M.; Bulat, V.; Majcen, K.; Dzapo, A.; Jezovita, J. *Coll. Antropol.* **2014**, 38, 1249-53.

only a few of them fulfill the requirements to be used in phototherapy. Thus, the development of new photosensitizers continues to be a current objective of research in the field of PDT.[23]

Subcellular localization of PSs: The efficiency of a photosensitizer for PDT is related to its subcellular localization.[24] Therefore, we first evaluated the cell internalization and subcellular localization of OPEs **3-OPE-18** and **3-OPE-28** in HaCaT (human keratinocyte cells) HeLa (human cervical cancer cells) and HEp-2 (human larynx cancer cells) cells was analyzed by fluorescence microscopy under UV excitation light (360-370 nm) (Figure 13A). Similar results were observed under blue light excitation (460-490 nm). The intracellular luminescent signals of **3-OPE-18** and **3-OPE-28** were observed after 6 h of incubation with $1 \cdot 10^{-6}$ M of both compounds.

Corresponding controls (without PSs incubation) of each cell type were also performed. In the three cell lines, after the incubation with PSs, an intense signal was visualized around the nucleus in a reticular pattern, as well as in a yuxtanuclear position. By contrast, control cells showed a very low signal, corresponding to mitochondrial autofluorescence. This indicated that signal emitted by treated HaCaT, HeLa and HEp-2 cells was due to the intracellular accumulation of the PSs, confirming the cellular uptakes of both compounds. In order to determine the places where PSs were located, cells were incubated with known markers for endoplasmic reticulum (ER-Tracker®) and Golgi apparatus (NBD®) (Figure 13B). ER showed a blue fluorescent signal

[23] Allison, R. R.; Downie, G. H.; Cuenca, R.; Hu, X. H.; Childs, C.J. H.; Sibata, C. H. *Photodiagn. Photodyn. Ther.* **2004**, *1*, 27−42. (b) Garland, M.J.; Cassidy, C.M.; Woolfson, D.; Donnelly, R.F. Designing, *Future Med. Chem*. **2009**, *1*, 667-691.

[24] (a) Soares, A.R.M.; Neves M.G.P.M.S.; Tome, A.C.; Iglesias-de la Cruz M.C.; Zamarrón, A.; Carrasco, E.; González, S.; Cavaleiro, J.A.S.; Torres, T.; Guldi, D.M.; Juarranz, A., *Chem. Res. Toxicol.* **2012**, *25*, 940-951. (b) Rello-Varona, S.;Gámez, A.;Moreno, V.;Stockert, J.C.; Cristóbal, J.;Pacheco, M.; Cañete, M.; Juarranz, A.; Villanueva, A. , *Int. J. Biochem. Cell. Biol*. **2006**, *38*, 23-OPE-183–2195.

under UV excitation light (λ = 360-370 nm) and Golgi apparatus emitted a green fluorescence under blue excitation light (λ = 460-490 nm).

Comparison of the images obtained after incubation with **3-OPE-18** and **3-OPE-28** with those obtained with the known probes for specific organelles, confirmed that compounds **3-OPE-18** and **3-OPE-28** are localized mainly in Golgi apparatus and endoplasmic reticulum. It is important to indicate that no differences were found in the intracellular localization of OPE-**3-OPE-18** or **3-OPE-28** in the three cell types.

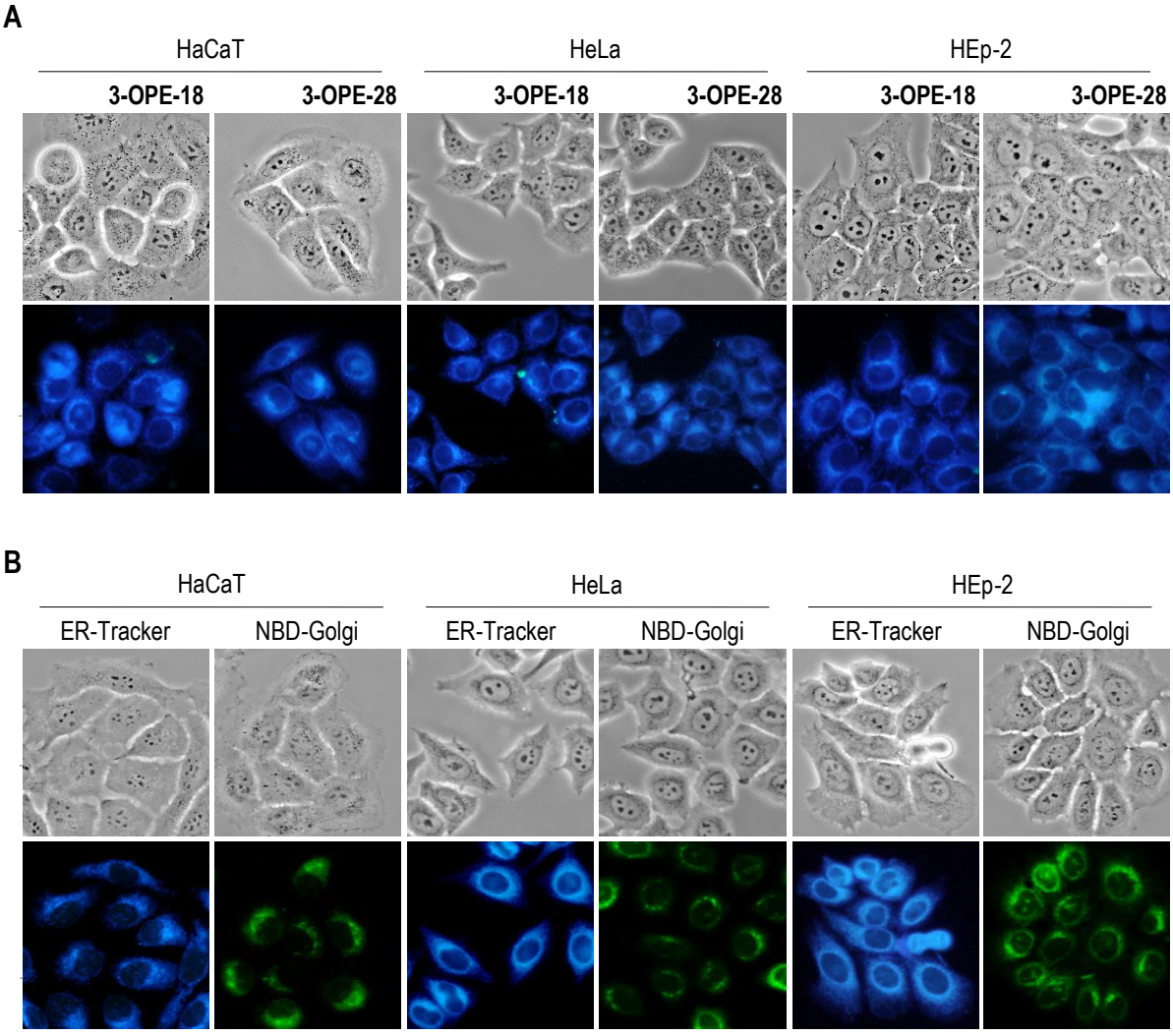


Figure 13

Effects induced by 3-OPE-18 and 3-OPE-28 – PDT on cell survival: The action of a PS on cells is related to the production of $^1O_2$ and/or other ROS generated after light irradiation.[25]

Therefore, we next evaluated the toxicity of compounds **3-OPE-18** and **3-OPE-28** first in the absence of light and then after UV irradiation. The toxicity experiments were performed by incubation of HaCaT, HeLa or HEp-2 cells with **3-OPE-18** or **3-OPE-28** (ranging from $3\cdot10^{-6}$to $2.5\cdot10^{-5}$ M) in the dark. In the absence of light the toxicity for both OPEs **3-OPE-18** and **3-OPE-28**, was dependent on the PS concentration (Table 2).

Thus, concentrations of $3\cdot10^{-6}$ and $5\cdot10^{-6}$ M of both PSs did not induce any relevant toxic effect (survival rates above 90% in all cell lines), and higher PS concentrations ($1\cdot10^{-5}$ and $2.5\cdot10^{-5}$M) caused a variable cell damage depending on the cell type (survival percentages between 60-85%).

The dark toxicity levels obtained for both OPEs **3-OPE-18** and **3-OPE-28** are similar to other reported PSs tested *in vitro*, including porphyrin derivatives and phtalocyanines.[26]

	HaCaT		**HeLa**		**HEp-2**	
[PS]	**3-OPE-18**	**3-OPE-28**	**3-OPE-18**	**3-OPE-28**	**3-OPE-18**	**3-OPE-28**
$3\cdot10^{-6}$ M	92.36 ± 1.6	91.45 ± 1.3	96.38 ± 1.6	96.61 ± 2.9	97.22 ± 1.4	97.73 ± 1.0
$5\cdot10^{-6}$ M	91.41 ± 2.4	90.93 ± 2.0	95.43 ± 1.7	93.52 ± 2.2	96.28 ± 2.0	95.72 ± 2.4
$1\cdot10^{-5}$ M	71.55 ± 3.2	70.23 ± 2.7	82.21 ± 2.5	76.89 ± 3.0	85.63 ± 2.9	80.07 ± 2.8
$2.5\cdot10^{-5}$ M	61.18 ± 1.8	59.72 ± 2.8	77.76 ± 2.7	66.65 ± 3.1	81.49 ± 1.9	70.19 ± 1.2

Table 2

[25] a) Barata, J.F.; Zamarrón, A.; Neves, M.G.; Faustino, M.A.; Tomé, A.C.; Cavaleiro, J.A.; Röder, B.; Juarranz, A.; Sanz-Rodríguez, F. *Eur. J. Med. Chem.* **2015**, *92*, 135-144. (b) Zhi, Y.-G.; Lai, S.-W.; Chan, Q. K.-W.; Law, Y.-C.; Tong, G. S.-M.; Che, C.-M. *Eur. J. Org. Chem.* **2006**, 3125–3139.

[26] (a) Henderson, T.J., *Anal. Chem*. **2002**, *74*, 191–198. (b) Demas, N. Crosby, G. A., *J. Phys. Chem.* **1971**, *75*, 991-1024.

We next evaluated the effect of UVA irradiation on the cell cultures, first without the presence of the OPEs. As can been seen in Table 3, none of the tested doses (ranging from 0.25 to 2 J/cm^2) induced substantial cytotoxic effects, the survival rates of HaCaT, HeLa and HEp-2 after UVA light irradiation (2 J/cm^2) were 97.2%, 94.0% and 95.1%, respectively.

UVA dose	**HaCaT**	**HeLa**	**HEp-2**
0.25 J/cm^2	98.76 ± 1.0	99.01 ± 0.9	98.11 ± 1.8
0.5 J/cm^2	98.33 ± 1.1	97.50 ± 2.0	98.42 ± 1.3
1 J/cm^2	97.92 ± 2.1	96.79 ± 1.7	97.33 ± 1.5
2 J/cm^2	97.2 ± 1.2	94.0 ± 2.3	95.2 ± 1.6

Table 3

According to the above illustrated results we selected concentrations of $3 \cdot 10^{-6}$ and $5 \cdot 10^{-6}$ M of both compounds and 0.25, 0.5 and 1 J/cm^2 of UVA light to evaluate the the synergic effect of the PSs compounds **18** and **3-OPE-28** plus UVA irradiation on the three cell lines. The photodynamic treatments, carried out by incubation with OPE-**3-OPE-18** or OPE-**3-OPE-28** for 6 h followed by UVA exposure, induced cytotoxic effects in HaCaT, HeLa and HEp-2, depending on the PS concentration and the UVA dose. Cell survival rates after PDT (Figure 14A), measured by MTT assay, were significantly decreased (P<0.01) in all the cell lines with respect to untreated controls. Viability after PDT was similar for both PSs, since no significant differences between **3-OPE-18** and **3-OPE-28** were found. However, HeLa cell extended to be slightly more sensitive to PDT than HaCaT and HEp-2. The LD50 dose, which corresponds to the treatment conditions that induced approximately a 50% of cell death, was reached with concentrations of $3 \cdot 10^{-6}$ of **3-OPE-18** or **3-OPE-28** and followed by 0.25 J/cm^2 of UVA radiation. The other tested conditions caused a lethality rate greater than 50%. In the case of **3-OPE-18**, values lower than 10% of cell survival

(LD90) were obtained after cell treatment with $2.5 \cdot 10^{-5}$ M and 2 J/cm^2 of UVA light (data not shown). Similar results were found for **3-OPE-28**. All these findings indicated that compounds **3-OPE-18** and **3-OPE-28** were not toxic for the cells in the absence of light at concentrations lower than $5 \cdot 10^{-6}$ M, confirming that the cytotoxic effect when UVA light was applied was due to the production of reactive oxygen species (ROS). These cytotoxic values are comparable with other

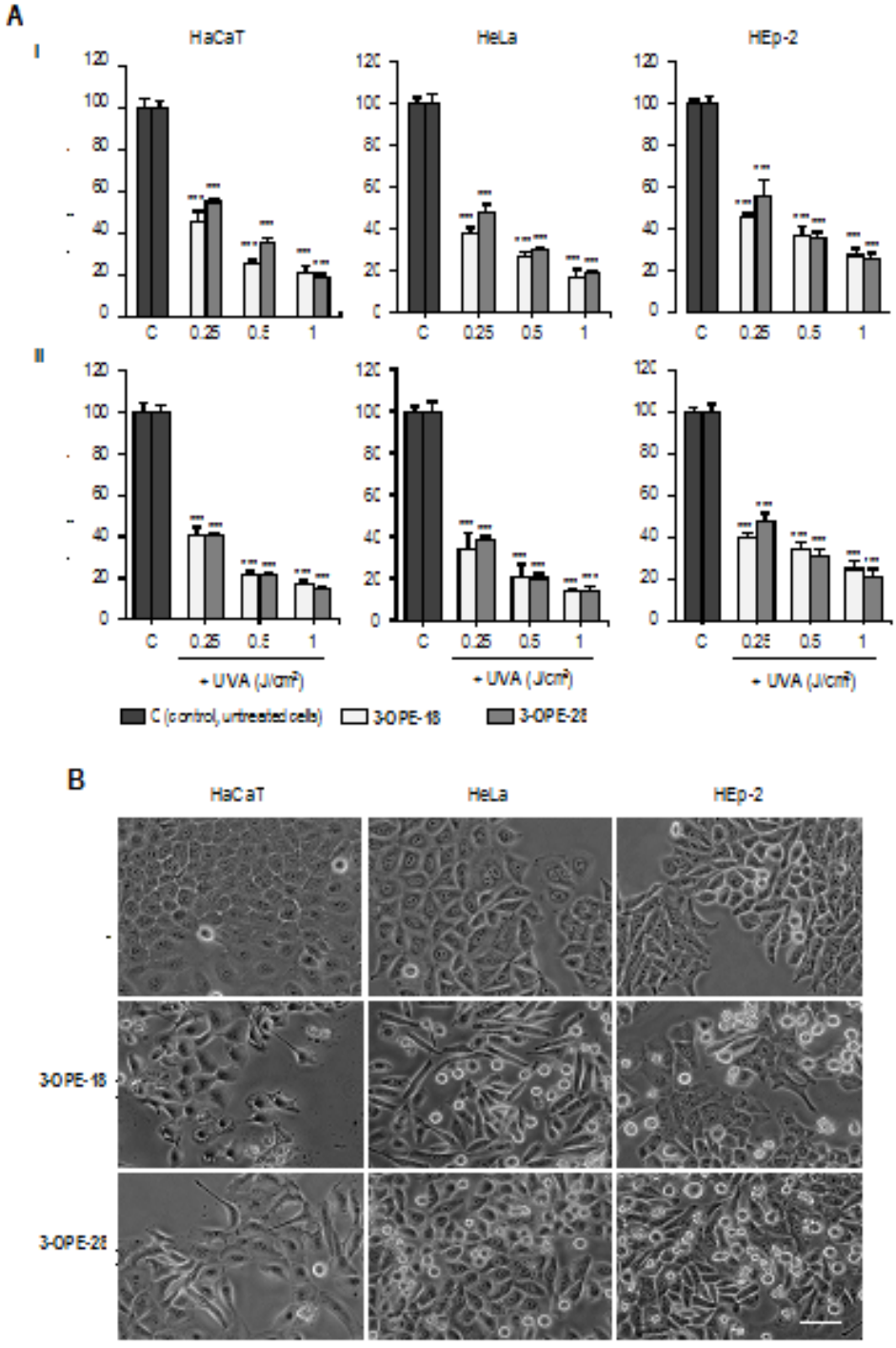


Figure 14

PSs used in PDT under UVA light irradiation, such as curcumin[27], hypericin or psoralen.[28] Both PSs reached a good photodynamic effect when combined with UVA light, but we did not observed selectivity for tumour HeLa and HEp-2 cells compared to non-tumour HaCaT cell line. The cell morphology was also analyzed 24 h after phototreatments, using phase contrast microscopy (Figure 14B).

This analysis revealed that **3-OPE-18** and **3-OPE-28**-based PDT induced notable changes in the morphology of HaCaT, HeLa and HEp-2 cells. These alterations were dependent on the treatment conditions (PS concentration and UVA light dose) and did not differ when **3-OPE-18** or **3-OPE-28** were used. Under a LD50 conditions, HaCaT cells showed cytoplasmic retraction and cell elongation, as well as the appearance of apoptotic (with membrane *blebbing*) and necrotic (with loss of membrane integrity) figures.

The tumoural cell lines (HeLa and HEp-2) also suffered retraction of their cytoplasm, cellular stretching and a strongly cytoplasmic vacuolization, which is indicative of cell degeneration processes. In addition, we noticed the apparition of a large amount of rounded cells, indicating a possible mitotic blockage, as well as a large number of cells detached from the substrate, floating on culture medium. All the changes observed confirmed the results obtained from cell viability assays.

Nuclear morphology was also evaluated 24 h after PDT by Höechst-33258 staining and fluorescence microscopy (Figure 15A-B). Nuclear retraction was observed in the three cell lines when subjected to LD50 dose of both phototreatments, in comparison to untreated cells, as well

[27] Dempster, D. N.; Morrow, T.; Quinn, M. F., *J. Photochem*. **1974**, *2*, 329-341.

[28] Rello, S.; Stockert, J.C.; Moreno, V.; Gámez, A.; Pacheco, M.; Juarranz, A.; Cañete, M.; Villanueva, A., *Apoptosis*. **2005**, *10*, 201-208.

as the appearance of apoptotic and necrotic morphologies. Furthermore, a great amount of mitotic nuclei was observed in HeLa and HEp-2 cell lines, most of them abnormal metaphases.

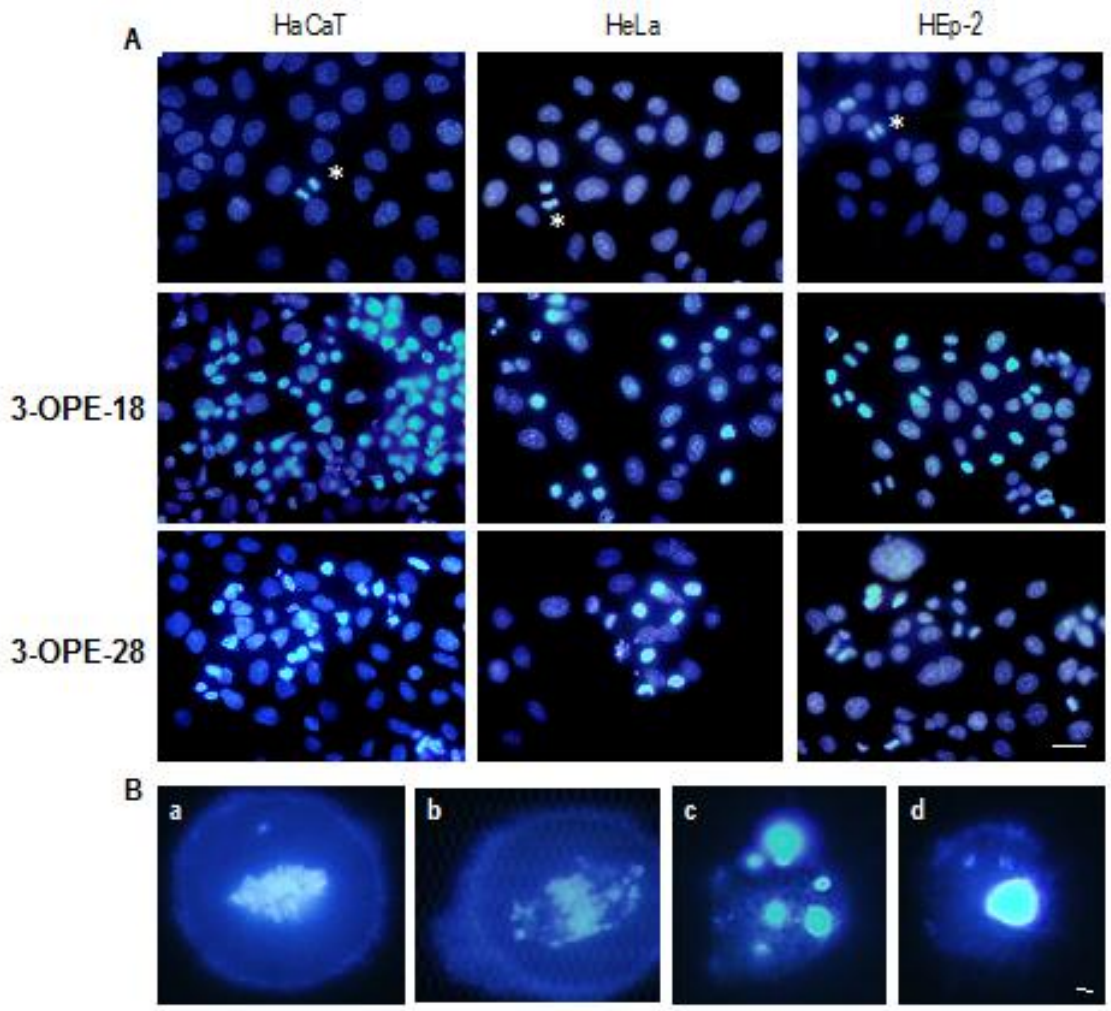


Figure 15

Nuclei morphology was observed 24 h after PDT using Höechst-33258 staining and fluorescence microscopy (in figure 15A). The controls (untreated cells) of the three cell lines displayed well defined interphasic and mitotic (*) nuclei. Nevertheless, when treated with OPE-**3-OPE-18** or OPE-**3-OPE-28,** PDT (LD50 dose), cultures showed an increment of apoptotic and necrotic nuclei. Also, in HeLa and HEp-2 cultures an increase of mitotic cells was observed (figure 15 B).

Details of normal (a) and altered (b) mitotic figures as well as apoptotic (c) and necrotic (d) cells are also shown.

Acridine Orange / ethidium bromide staining: The cytotoxicity of LD50 of **3-OPE-18** and **3-OPE-28** in PDT was also evaluated using acridine orange/ethidium bromide staining (AO/EtBr) (Figure 15). This method allowed us to distinguish between viable, rounded, apoptotic and necrotic cells. As shows Figure 16A, the PDT induced evident alterations in the morphology of HaCaT, HeLa and HEp-2 cells, in comparison with their control counterparts. These morphological changes were similar for both PSs. After PDT, HaCaT cultures showed an increase of apoptotic and necrotic cells. In HeLa and HEp-2 cultures, besides apoptotic and necrotic figures, a large number of rounded cells densely stained as green, were observed. In order to determine the exact percentage of each cellular condition, a cell counting was performed.

The percentage of cells in each established condition was similar for **3-OPE-18** (Figure 16B) and **3-OPE-28** (Figure 16C) phototreatments. HaCaT cells showed a significantly higher proportion of apoptotic and necrotic figures in comparison with tumor cell lines. By contrast, in the case of HeLa and HEp-2, cell counting revealed a significant increase ($P<0.01$) of rounded cells as compared to HaCaT and to its control counterparts. All of these results suggest that photodynamic treatment could be triggering a mitotic blockage in tumor cell lines, while in HaCaT mainly induce apoptotic processes.

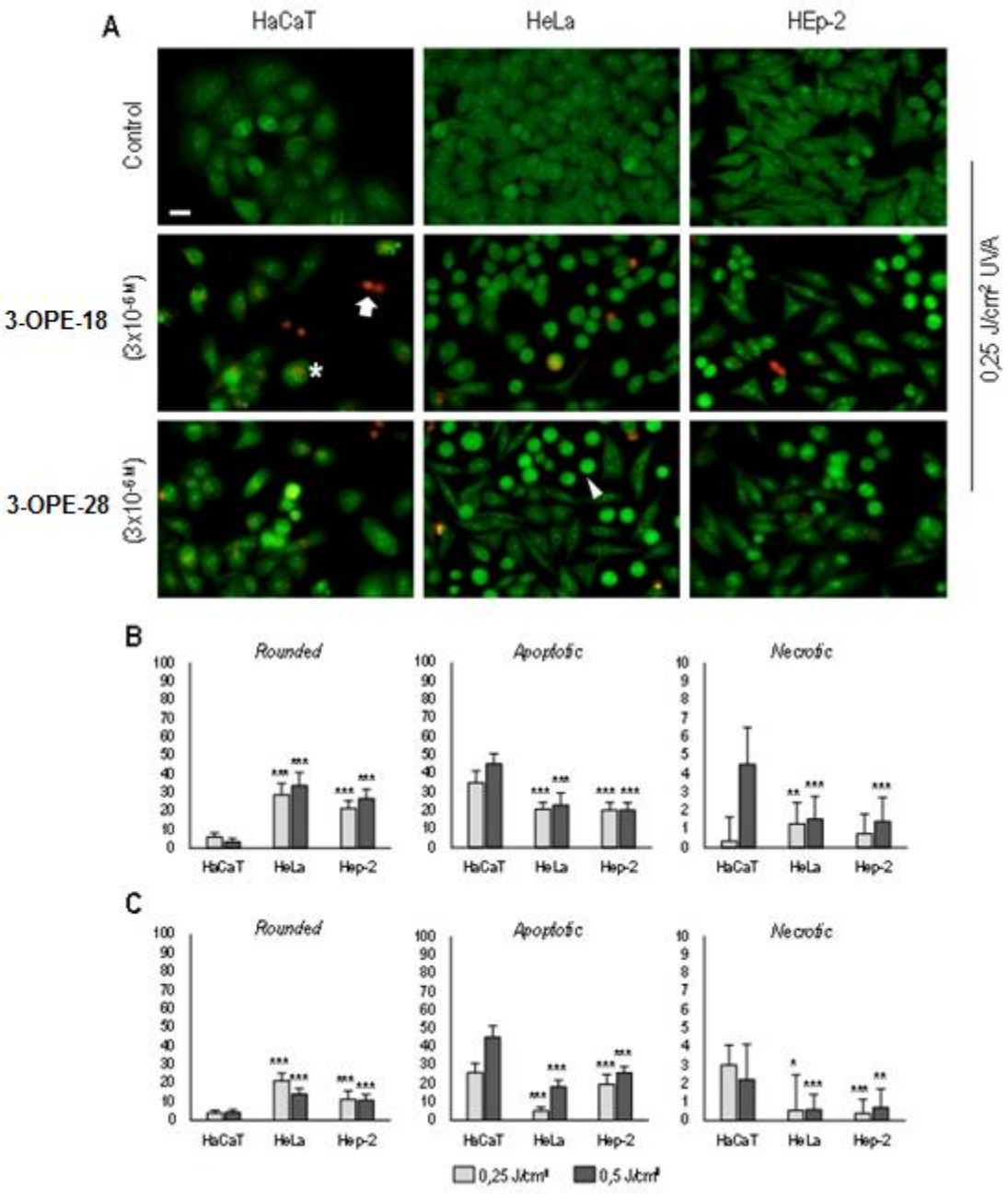


Figure 16

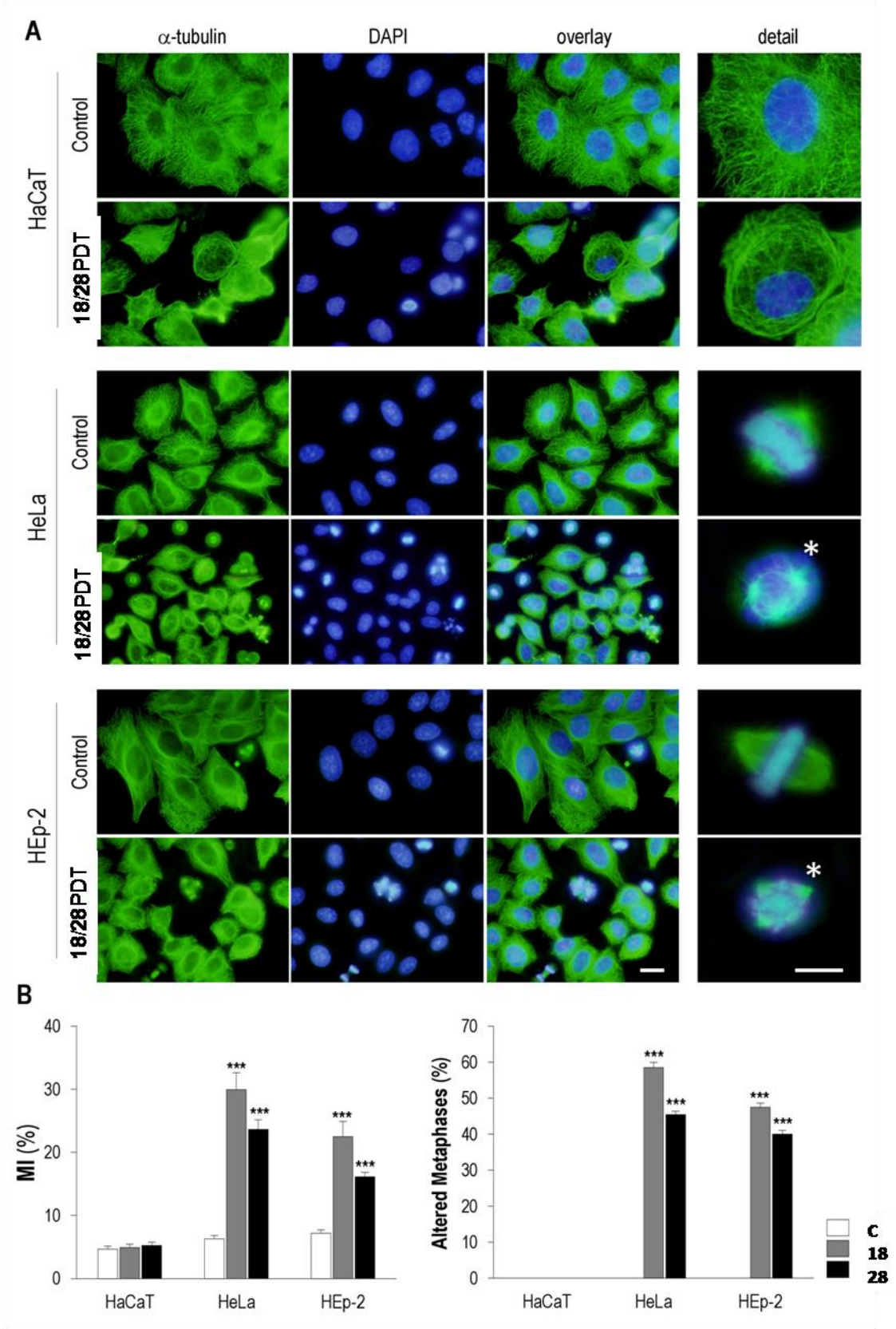
Cytoskeleton of microtubules as a target for 3-OPE-18 or 3-OPE-28-PDT: To get insight into the mechanisms by which PDT using **3-OPE-18** or **3-OPE-28** as PSs reach their effects on HaCaT, HeLa and HEp-2 cells, we also evaluated the changes induced by PDT in the cytoskeleton of microtubules (MTs) (Figure 16). MTs are cytoskeletal polymers essential for cell structure, cell division and intracellular transport and their role in tumor progression makes them

a key target for anti-cancer therapies, including PDT.[29] As shown Figure 17A, the interphasic HaCaT, HeLa and HEp-2 cells showed a well-developed MT network and no alterations were observed in the mitotic spindle of dividing cells. However, cells showed a clear increase in microtubular changes 24 h after phototreatments with LD50 doses.

Most HaCaT cells retracted their cytoplasm and thus showed an altered and disorganized MT network. Moreover, apoptotic morphologies were also observed. With respect to HeLa and HEp-2 cells, in addition to the described alterations, there was also an increase in the number of dividing cells and many of them showed modifications of the spindle apparatus, such as the presence of extrapoles (three and four-poles spindles) and the consequent abnormalities in the distribution of chromosomes in the metaphase plate.

In order to determine also the percentage of cells in possible mitotic blockage, mitotic index was quantified 24 h after **3-OPE-18** and **3-OPE-28** phototreatments (Figure 17B). As shown in Figure 17A, after **3-OPE-18** and **3-OPE-28**-PDT in LD50 conditions, mitotic index of HeLa and HEp-2 was significantly higher as compared to untreated (control) cells. By contrast, mitotic index of HaCaT cells did not change after PDT. The percentage of mitotic cells with abnormal metaphase plates was also calculated (Figure 17B). In the same way, PDT of HeLa and HEp-2 cells subjected to LD50 of **3-OPE-18** or **3-OPE-28** presented a significantly higher percentage of altered metaphases in comparison with untreated cells, while phototreatments did not induce aberrant metaphases in HaCaT cells. These findings suggest that MTs could be a target of phototreatments with compounds **3-OPE-18** and **3-OPE-28** plus UVA light, which cause the disorganization and collapse of MTs network and, in the case of tumoral cell lines, also the incorrect functioning of mitotic apparatus and thus the appearance of aberrant metaphases.

[29] (a)Wakamiya, T.; Matsubara, Y.; Yoshida, Z.-i., *J. Am. Chem. Soc.* **2005**, *127*, 9332-9333 (b) Liu, K.; Liu, P.C.; Liu, R.;Wu, X. Med., *Sci. Monit. Basic. Res*. **2015**, *21*, 15-20.


A
α-tubulin
DAPI
overlay
detail
HaCaT
Control
18/28PDT
HeLa
Control
18/28PDT
HEp-2
Control
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*
*
B
MI (%)
40
30
20
10
0
***
***
***
***
HaCaT
HeLa
HEp-2
Altered Metaphases (%)
70
60
50
40
30
20
10
0
***
***
***
***
HaCaT
HeLa
HEp-2
C
18
28

Figure 17

In conclusion, compounds **3-OPE-18** and **3-OPE-28** are susceptible to be excited by UVA light and produced a high photodynamic effect causing tumour cell death even with low singlet oxygen production. This photodynamic effect seems to induce damage in the MTs network of tumour cells, triggering a blockage in the metaphase of cell cycle, which can lead to cell death. The glucose-ended OPEs tested have been able to produce singlet oxygen with moderated efficiencies. The study presented here provides the first use of OPEs as photosensitizers and could be a starting point to extend the application of these luminescent oligomers in photodynamic therapy.

Synthetic modifications of OPEs

Chapter 3

In order to modulate and enhance the biological and photo-physical properties of our OPE glicosides (Cap. 2) and to study their supramolecular behavior, we thought to act some structural modification to their structure, following four different strategies:

1. Changing sugar to modulate biological properties;
2. Modifying the linear structure to study the aggregation;
3. Inserting a bodipy *core* to modulate photo-physical properties;
4. Building a C-2 symmetric structure containing an enantiomerical pure functions.

The preliminar synthetic results of these modifications are reported in this chapter.

3.1 Synthesis of galacto-OPEs

The biological results obtained with our gluco-OPEs on different cell lines were illustrated in Chapter 2; taking into account that a different carbohydrate linked to our fluorescent probes could be determining in cell selectivity, we thought to insert a different sugar at the end of our OPE chain, with the final aim to observe how it could influence the biological behavior.

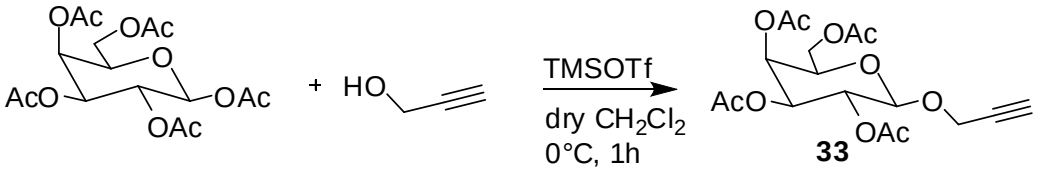
The chosen monosaccharide was β-galactose: in fact it is known that the galacto-derivatives easily link to lectines, proteins presents on the cellular membrane, helping the cell internalization[1].

Having in mind that, as shown in Chapter 2, from a biological point of view the more interesting synthesized molecule was **18**[2], we planned to synthesize the galacto-analogous using the same convergent strategy.

[1](a) Collins,B. E.; Paulson; J.C.; *Curr. Opin. Chem. Biol.*, **2004**, 8, 617-625. (b) Lundquist, J. J.; Toone, E.J.; *Chem. Rev.*, **2002**, 102, 555-578.

[2] Barattucci, A.; Deni, E.; Bonaccorsi, P.; Ceraolo, M. G.; Papalia, T.; Santoro,A.; Sciortino, M. T.; Puntoriero, F., *J. Org. Chem.* **2014**, *79*, 5113−5120.

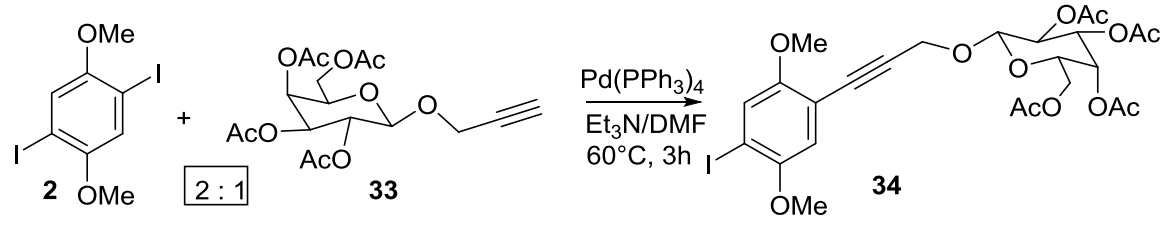
The first step was the synthesis of **33**,the properly galactose building block. It was obtained by reaction from commercial 1,2,3,4,6-penta-*O*-acetil-*β*-D-galactopyranose in dry $CH_2Cl_2$and propargylalchol, in presence of trimethylsilyltriflate (TMSOTf) [3](Scheme 1).



Scheme 1

The addition of TMSOTf must be carried out slowly and at controlled temperature (0°C) to avoid the formation of side products. Compound **33** was obtained by chromatographic purification as a white crystal in very good yields (78%).

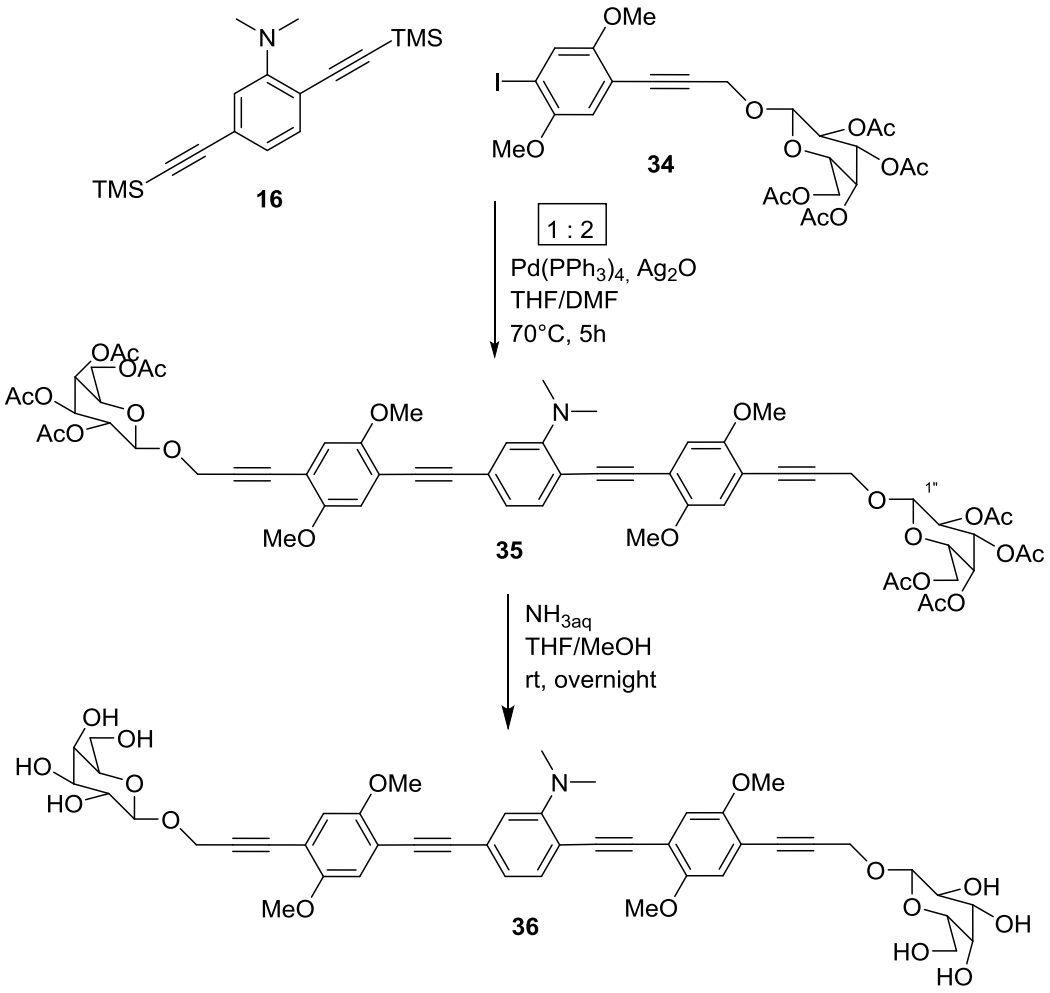
Once obtained **33**, it was possible build the appropriate galacto-monoderived, corresponding to gluco-one **4** (Chapter 2, scheme 4), that could be used in the cross coupling reaction with the dimethylamino *core* **16** (chapter 2, scheme 10). Compounds **33** and **2**(chapter 2, scheme 2) were reacted (scheme 2) in the same molecular ratio (**2 :33**= 2.1:1) and reaction conditions seen for the preparation of **4**. After 3 hours the reaction was complete and compound **34** was obtained by chromatographic separation as a white solid.



Scheme 2

[3]Giovenzana, G. B.; Lay, L.; Monti, D.; Palmisano, G.; Panza, L. *Tetrahedron***1999**, *55*, 14123-14136.

As for **17**, the synthesis of compound **35** was reached in five hours thanks to the Ag_2O-modified Sonogashira reaction (scheme 3).[4]



Scheme 3

Deacetylation[5] of **35** occurred using an excess of aqueous ammonia in THF/MeOH at room temperature and led to quantitative formation of **36**. The acetamide formed was removed by evaporation in *vacuo* and by washings with MeOH; **36** was obtained as a brilliant yellow solid.

Studies are underway to determine how the presence of galacto- substituent could modulate the biological activity in photodinamic treatments against tumor cells.

[4] Mori, A.; Kondo, T.; Kato, T.; Nishihara Y. *Chem. Lett.* **2001**, 286-287.

[5]Hasegawa, T.; Numata, M.; Okumura, S.; Kimura, T.; Sakurai, K.; Shinkai, S. *Org. Biomol. Chem.***2007**, *5*, 2404-2412.

3.2 Synthesis of trifunctionalized OPEs

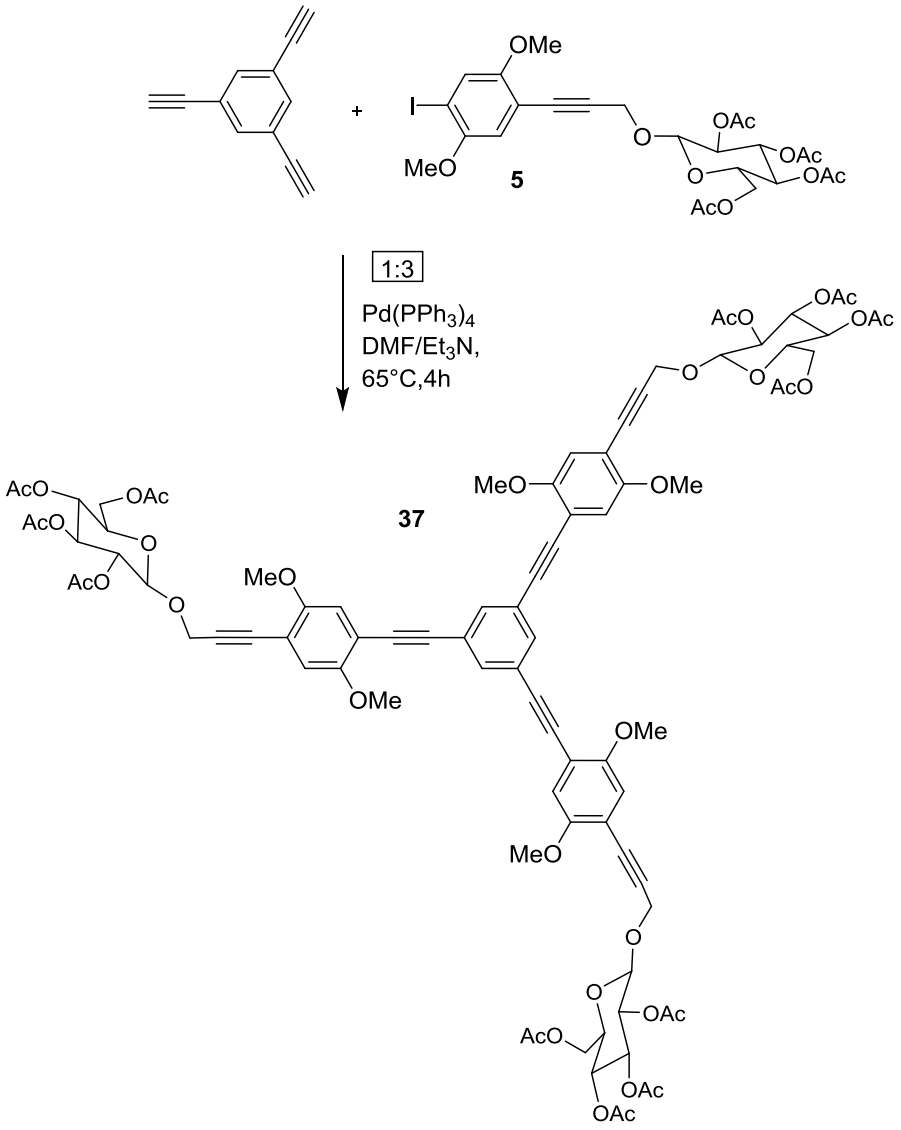
It is widely known that oligophenyleneethynylenes have the ability to self-assemble into different supramolecular structures[6] in different solvent mixtures. Furthermore it's known in literature that the introduction of enantiopure moieties (often derived from biomolecules, e.g., monosaccharides, aminoacids or nucleosides) as substituents on the conjugated skeleton of OPEs or PPEs (poliphenyleneethynylenes, cap.1) significantly affects their solid state organization and opens intriguingpossibilities in enantioselective sensing applications[7]. These results opened the possibility to use OPEs in nanomaterial field.

With these prerequisites, we thought that our OPEs, containing both hydrophobic (linear OPE chain) and hydrophilic (sugar terminations) moieties could be very appealing in aggregation studies. Having in our hands linear and di-sugar ended wires, we thought to insert a third hydrophilic arm on our systems, with the double intention to help the water solubility and reinforce the intermolecular interactions (e.g. hydrogen bonding between the sugar moieties and pi-staking between the aromatic counterparts) for possible applications in studies of supramolecolar aggregation.

The synthetic strategy was very intuitive and easy to realize: as a central trifunctionalized *core* commercial 1,3,5-triethynyl benzene was used: cross-coupling with mono derivative **4** in dry DMF (scheme 4), gave **37** after 4 hours and in good yields (60%).Chromatographic separation is indispensable to purify **37**from other undesidered products, coming from dimerization of mono- and disubstituted compounds, anyway formed in little percentages.

[6]a) F. Garcia, L. Sanchez, *J. Am. Chem. Soc.***2012**, 134, 734 –742 (b) F. Garcia, F. Aparicio, G. Fernandez, L. Sanchez, *Org. Lett.***2009**, 11, 2748 –2751 (c) K. Yoosaf, A. Belbakra, N. Armaroli, A. Llanes, Pallas, D. Bonifazi, *Chem. Commun.***2009**, 2830 –2832

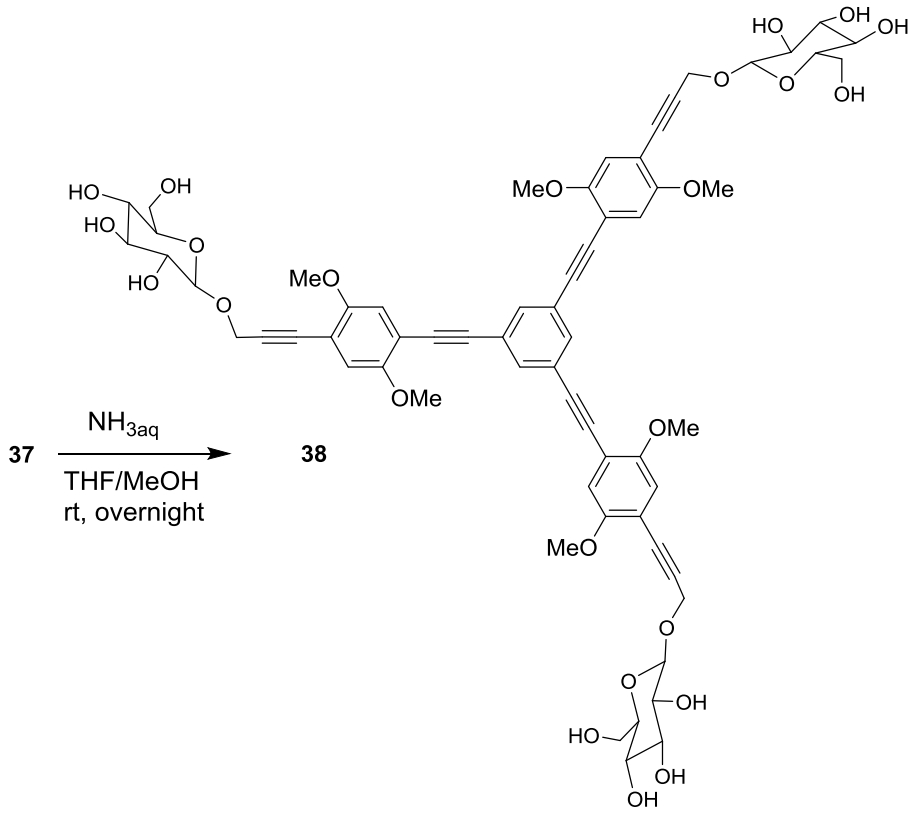
[7](a) Kushon, S. A.; Ley, K. D.; Bradford, K.; Jones, R. M.; McBranch, D.; Whitten, D. *Langmuir***2002**, 18, 7245−7249 (b) Torsi, L.; Farinola, G. M.; Marinelli, F.; Tanese, M. C.; Hassan Omar, O.; Valli, L.; Babudri, F.; Palmisano, F.; Zambonin, P. G.; Naso,*F. Nat. Mater.***2008**, 7, 412−417.



Scheme 4

Treatment of compound **37** with $NH_{3aq.}$ in a mixture of MeOH/THF at rt, after 16 hours afforded **38** quantitatively (Scheme 5), interesting for the presence of three sugar substituents.

Aggregation studies in organic solvents and in water will be performed on compound **37** and on its respective deacetyled **38**, as for the linear analogous.



Scheme 5

3.3 Synthesis of BODIPY-OPEs

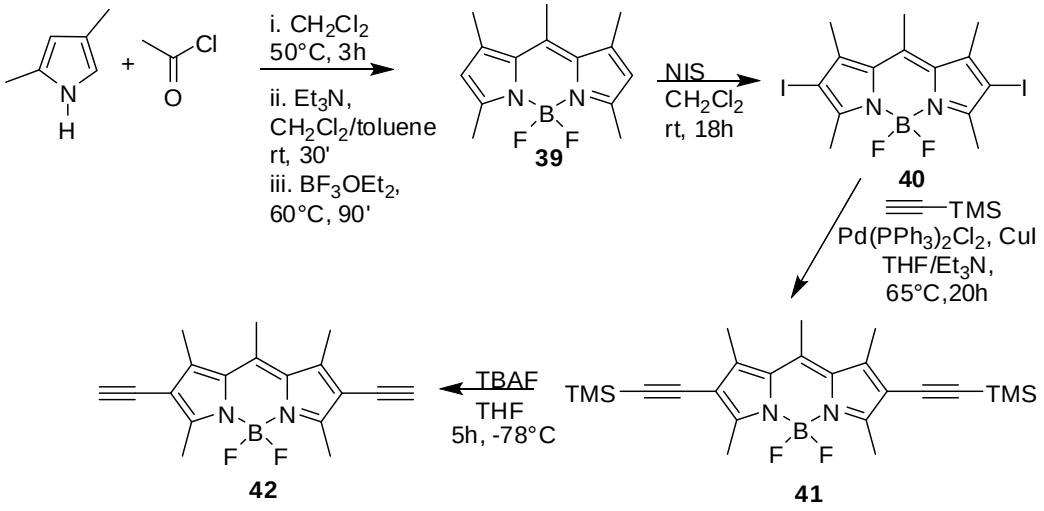
Thanks to their photophysical behavior, including the production of oxygen singlet by irradiation with UV-Vis light, our synthesized OPEs demonstrated to act with very good results in photodynamic treatments in HEp-2 and Hela tumor cells (chapter 2). It is also known that,in biological field, both for bio-imaging (emission interceptable with fluorescence detectors) and for photo-dynamic treatment against not epidermal cancers, more penetrating radiations, like the infrared ones,have to be used.[8]

Herein, our modification is aimed to the synthesis of a new OPE which can absorb and emit radiations with higher wavelength, near to biological 'red-window': for this reason, we thought to

[8] (a) Thivierge, C.; Han, J.; Jenkins, R. M.; Burgess, K.; *J. Org. Chem.*, **2011**, 76, 5219; (b) Xiao, Y.; Zhang, D.; Qian, X.; Costela, A.; Garcia-Moreno, I.; Martin, V.; Perez-Ojeda, M. E.; Banuelos, J.; Gartzia, L.; Arbeloa, I. L.; *Chem. Commun.*, **2011**, 47, 11513.

introduce in the OPE's skeleton a bodipy (BoronDiPyrromethene) *core* which, thanks to extraordinary photo physical properties,[9] could considerably shift the wavelength of adsorption and emission of our molecules. The choice of bodipy wasn't casual; in fact, the group in which I developed my thesis, is being involved for several years also in the synthesis of glycoconjugated bodipy[10] and in their application in bio-imaging[11]

The first step was the synthesis of a proper bodipy *core*, containing two reactive arms available to react with the monoderived **4**. Scheme 6shows the complete synthetic route that leads to the formation of bodipy **42**.



Scheme 6

The first reaction is the one-pot formation of bodipy **39**:[12] Condensation of acetyl chloride and 2,4-dimethyl-pirrole at 50°C followed by the addition of triethylamine and BF_3OEt_2, afforded cyclization to bodipy **39**, obtained as a red wine colored solid and with a48% yield. NIS iodation of

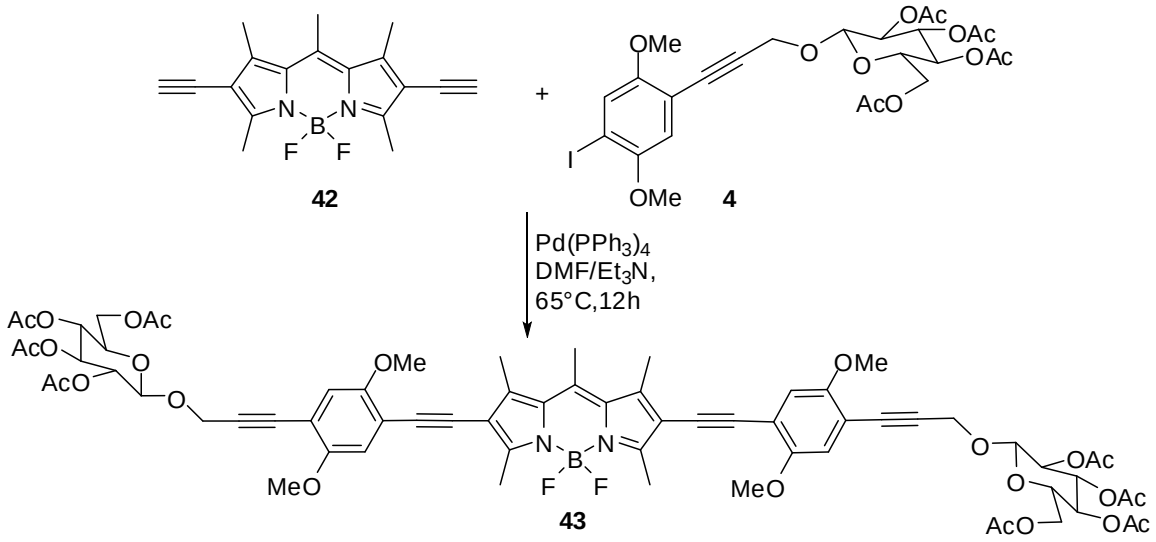
[9]Ulrich, G.; Ziessel, R.; Harriman, A.; *Ang. Chem. Int. Ed.* **2008**, *47*, 1184-1201

[10] Papalia, T.; Barattucci, A.; Aversa, M. C.; Campagna, S.; Puntoriero, F.; Bonaccorsi, P.; *Chem. Commun.* **2012**, 48, 10550–10552

[11] Papalia, T.; Siracusano, G.; Colao, I.; Barattucci, A.; Aversa, M. C.; Serroni, S.; Zappala, G.; Campagna, S.; Sciortino M. T.; Puntoriero, F.; Bonaccorsi, P.; *Dyes Pigments* **2014**, 110, 67.

[12]Florian, A.; Mayoral, M. J.; Stepanenko, V.; Fernandez, G. *Chem. Eur. J.* **2012**, *18*, 14957-14961

39 at the free 2 and 6 positions[13] gave quantitatively **40** as a red solid. Compound **40** was functionalized by cross coupling reaction in presence of a large excess of commercial ethynyltrimethylsilane: this implicates the double insertion of two ethynyl groups at the borondipirromethenic *cor*e. This already known reaction[14] was performed using classical Sonogashira conditions: Pd(II)/CuI in dry THF and Et_3N, with the obtainment of **41** in71% yield. Finally, by treatment of **41** at -78° and under Argon with TBAF (tetrabutylammonium fluoride), the typical reactive for the cleavage of a C-Si bond, –TMS groups were removed to give **42** in very good yields (73%).



Scheme 7

Copper-free Sonogashira coupling was used for the assembly of **42** and **4**, in dry DMF and Et_3N and $Pd(PPh_3)_4$ as catalyst: the formation of bodipy-OPE **43** happened in 12 hours reaction, a longer reaction time compared to the other Sonogashira couplings (2.1, 3.1, 3.2): this is probably due to the lower reactivity of the *core* on respect to the electron-rich aromatics used until now.

[13]Bonnier, C.; Machin, D. D.; Abdi, O.; Koivisto, B. D. *Org Biomol Chem.* **2013**, *11*, 3756-3760
[14]Lin, H.-Y.; Huang, W.-C.; Chen, Y.-C.; Chou, H.-H.; Hsu, C.-Y.; Lin, J. T.; Lin, H.-W. *Chem. Commun.* **2012**, *48*, 8913–8915

For an acceptable success of this reaction, it was important to work in strictly anhydrous conditions, particularly keeping attention to the dryness of solvents. The formation of **43** was confirmed only by ^{1}H-NMRspectrum (Figure 1) of the little quantitative of isolated compound, butit was not completely characterized because of the very low yields of reaction. For this reason, studies arebeing performed to optimize the final synthetic step.

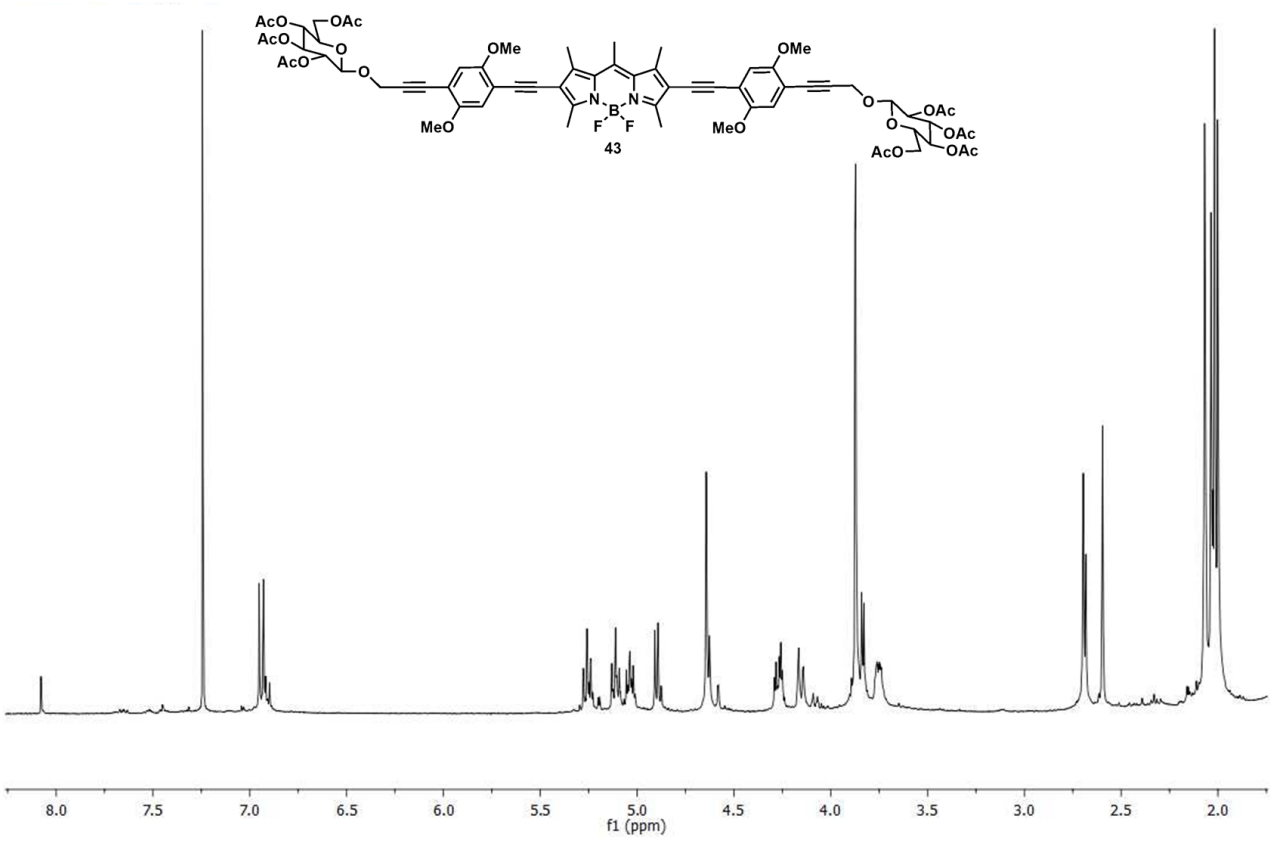


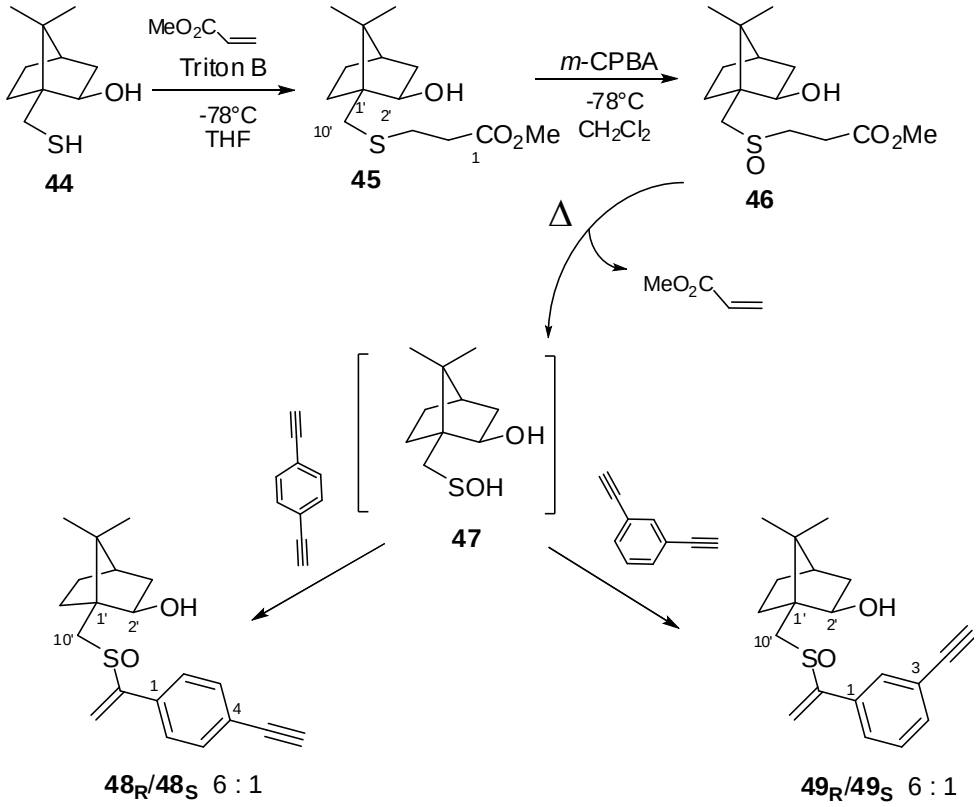
Figure 1

3.4 Synthesis of Sulfoxides-OPEs

The group in which I developed my PhD thesis has got a great experience on sulfoxides in asymmetric synthesis:for this reason it was envisaged the possibility of using phenylene-ethylene units in the construction of C_2-symmetric molecular compounds containing enantiomerically pure

sulfinylfunctions. In particular, the isoborneolsulfinyl moiety appeared a significant chiral controller and an interesting residue.[15]

For this purpose, it was decided to combine the classical synthetic procedure of OPE preparation, which involves the Sonogashira cross-coupling, with the well-known sulfenic acid/alkyne syn-addition to obtain sulfinyl derivatives in a stereo controlled manner.[16]



Scheme8

Treatment of thiol **44** with Triton B (benzyl(trimethyl)ammonium hydroxide) gave are active thiolate that immediately reacted, at -78° and in anhydrous THF, with methyl acrylate to afford quantitavely sulfide **45**, which was stereoselectively oxidized by *m*-CPBA (m-chloroperoxybenzoic acid) to a mixture of sulfoxide epimers **46**, new precursors of transient sulfenic acid**47** (Scheme 8).

[15] (a) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P.; Faggi, C.; Gacs-Baitz, E.; Marrocchi, A.; Minuti, L.; Taticchi, A.; *Tetrahedron* **2005**, 61, 7719; (b) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P.; Caruso, F.; Giannetto, P.; *Arkivoc* **2004**, 79; (c) Adams, H.; Jones, D. N.; Aversa, M. C.; Bonaccorsi, P.; Giannetto, P.; *TetrahedronLett.* **1993**, 34, 6481

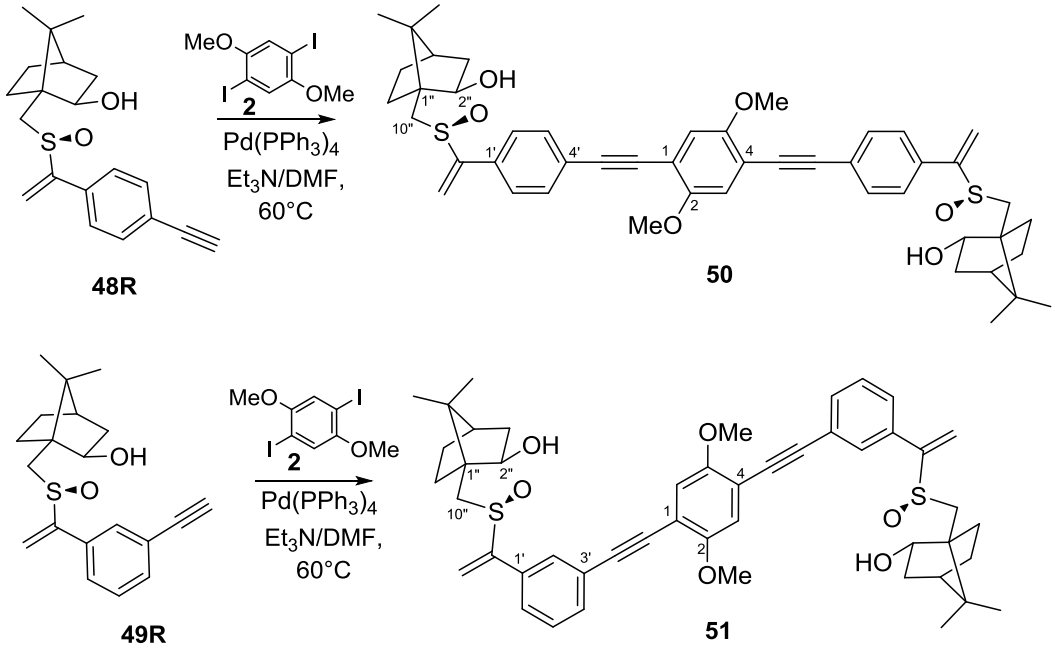
[16] Barattucci, A.; Aversa, M. C.; Deni, E.; Papalia, T.; Bonaccorsi, P.; *Helvetica Chimica Acta* **2014**, 97 1237

To test the best thermolysis conditions, **46** was heated in different solvents (toluene, 1,4-dioxane,1,2-dichloroethane (DCE), THF) at their respective reflux temperature, in thepresence of an excess (1 :6) of two different dialkynes, 1,3- or 1,4-diethynylbenzene.

While only decomposition products were obtained in toluene and dioxane, **47** was efficiently generated in situ in dichloroethane (DCE) at reflux and trapped by 1,3-or 1,4-diethynylbenzene to give **48** and **49** in excellent yields (>70%).

The high diastereo selectivity of both additions (Scheme 8) is ascribable to the preferred conformation adopted by **47**, due to a strong intramolecular H-bond between the sulfenic and the -OH functions.

The two couples of epimers **48R/48S** and **49R/49S** were easily separated by column chromatography and the four compounds were obtained in enantiomerically pure form.



Scheme9

The results of cross-coupling reactions of**48R** or **49R** with 1,4-diiodo-2,5-dimethoxybenzene **2**in DMF solution, and in the presence of a large excess of Et_3N and a catalytic amount of $Pd(PPh_3)_4$ are

outlined in Scheme 9. Despite the large excess of base, no racemization at the S-atom occured, and enantiomerically pure C_2-symmetric bis-sulfoxides **50** and **51**were obtained as sole products in high yields.

An inspection of the ^{1}H-NMR spectra of **48R**, **48S**, and **50**, as for **49R**, **49S**, and **51**, was useful to confirm the configurations at the S-atom. Altogether the simplicity of the ^{1}H-NMR spectra of **50** and **51** confirms their C_2 symmetry (figure 2).

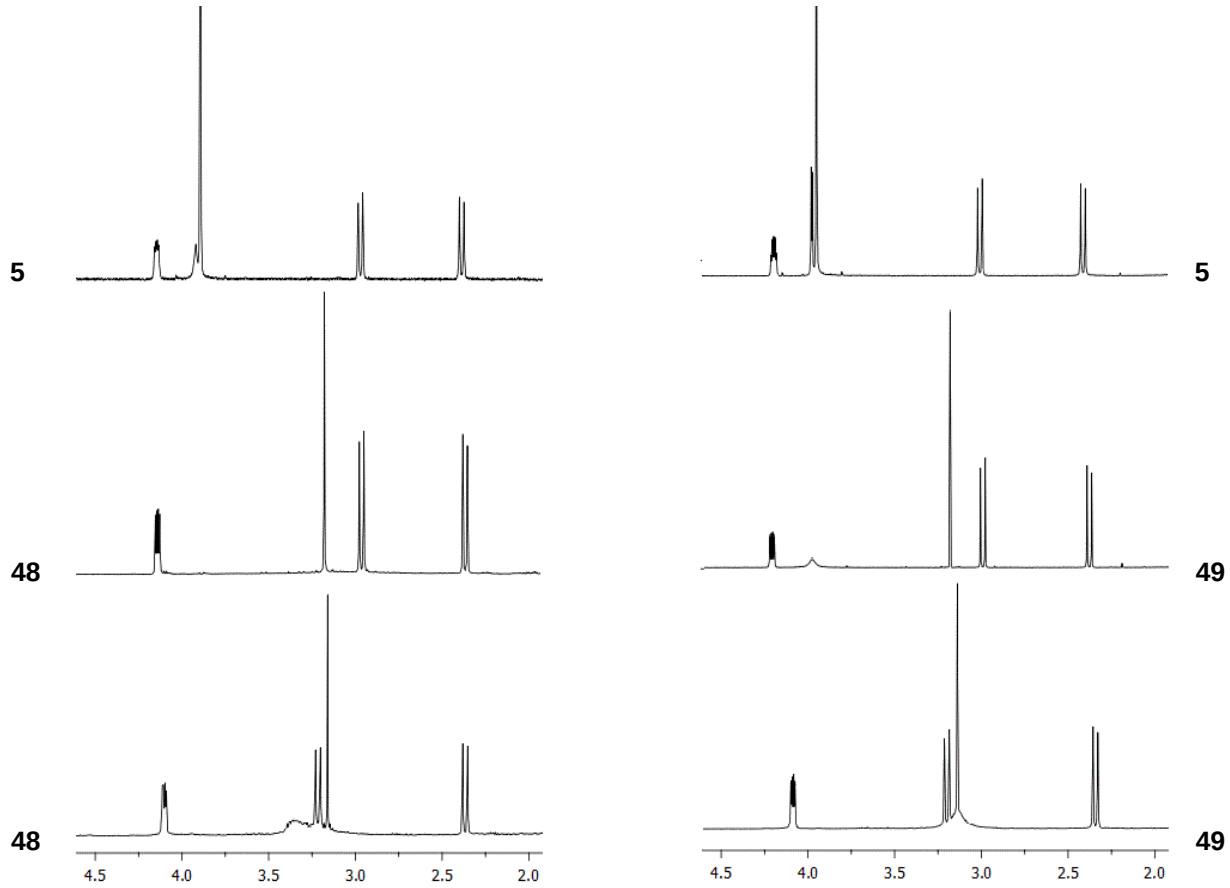


Figure 2

Synthesis of Azobenzene containing

Oligo(Phenylene)Ethynylenes

Chapter 4

Azobenzenes are compounds with the capacity to adsorb energy and use it for a molecular movement: the cis-trans isomerization. This process is a reversible transformation between two geometrical isomers with different absorption spectra. This transformation occurs as a consequence of an external input, like light or heat. The *trans (E)* isomer is more stable (~14 Kcal mol-1 (0.6 eV)) than the *cis (Z)* one. The energy barrier necessary to transform the trans to *cis* isomer is around is ~23 Kcal mol-1 (1.0 eV**)**. In fact, in the dark and at room temperature, the predominant isomer is the *trans* one.

When a solution containing the azobenzene is irradiated at a fixed wavelength, the process of photoisomerization *trans→cis* starts (figure 1). This process leads to a notable change in the dipolar moment and in the photophysical properties of the azobenzene. The photoisomerization can be followed by Uv/Vis because the absorption spectra of *trans* and *cis* are different.[1] Other techniques, mainly NMR, could also be applied to follow the photoisomerization process .

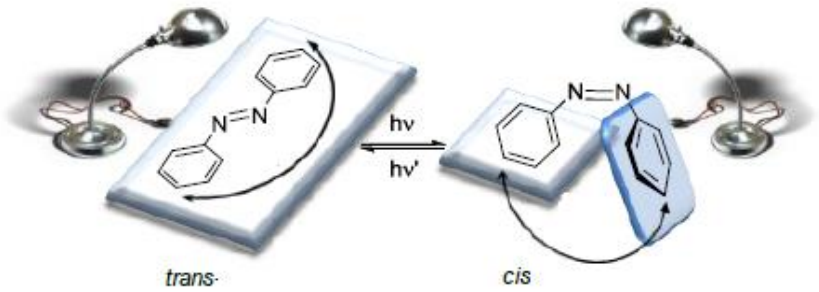


Figure 1

[1] a) Rau, H.; *Photochromism, Molecules and Systems*; **1990**, 165. b) Suginome, H.; *CRC Handbook of Organic Photochemistry and Photobiology*; **1995**, 824.

The UV/Vis spectroscopy is the technique more frequently used to study the photoisomerization. In general, in the adsorption spectrum, both isomers present two characteristic bands corresponding to the electronic transitions π→π* and n→π*. The π→π* transition is in the near UV (300-400nm), the n→π* is in the visible field and it's responsible of the typical color of the azo-systems.

The absorption spectra of the isomers *E* (*trans*) and *Z* (*cis)* have these typical features[2]:

- The *trans* (*E*) isomer: the band π→π* is more intense, with a molar extinction coefficient (ε) ~ 2-3 x 104 M-1 cm-1. The second band (n→π*) is less intense (ε ~ 400 M-1 cm-1) because it corresponds to a forbidden transition (**Figura 2**, blue line).
- The *cis* (*Z*) isomer: the band π→π* is slightly shifted toward lower wavelengths (hypsochromic effect) and the intensity is lower (ε ~ 7-10 x 103 M-1 cm-1). On the other hand, the transition n→π* (380-520 nm) absorption shows a higher intensity (ε ~ 1500 M-1 cm-1) compared to *trans* isomer (**Figura 2**, pink line).

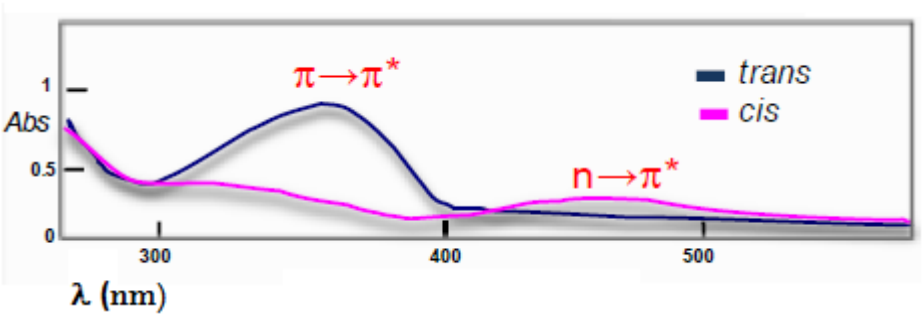


Figure 2

For the synthesis of azobenzenes, the most frequently used method is the azoic coupling, that involves diazonium salts.[3] Other procedures, like the Mills reactions, the reductive coupling of

[2] N. Tamai, H. Miyasaka, *Chem. Rev.* **2000**, *100*, 1875-1890.
[3] I. Szelle, H. Zollinger, *Top. Curr. Chem*. **1983,** *112*, 1-66.

nitro compounds and the oxidation of anilines also allow the synthesis of aromatic azo-compounds.[4](Figure 3).

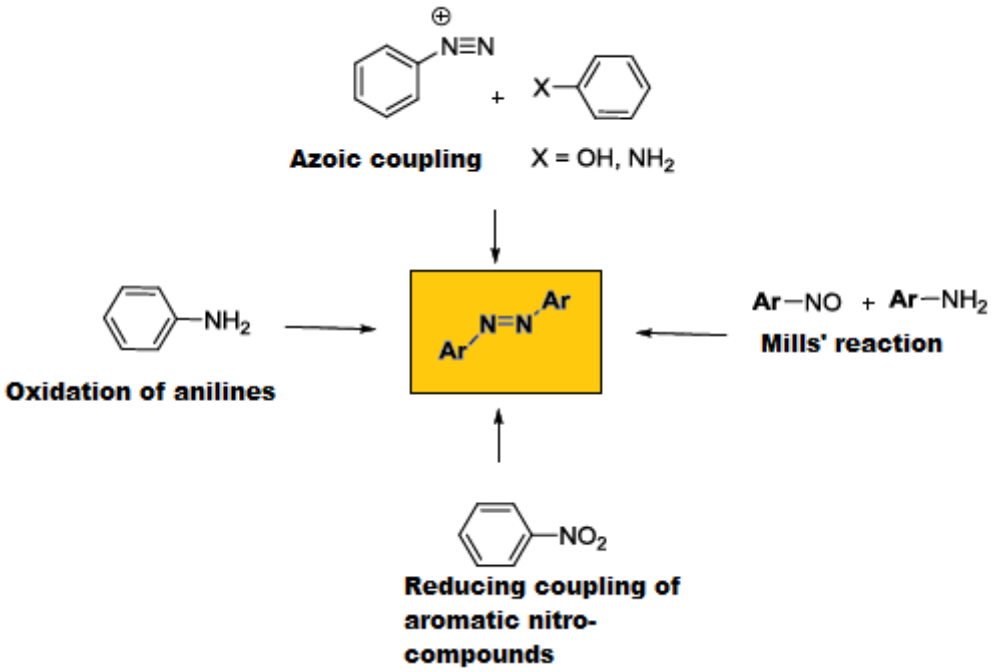


Figure 3

4.1 Synthesis of OPEs with an azobenzene core

The group of prof. M. C. Carreno, in which I spent a great part of my PhD, is specialized in the synthesis of enantiopure sulfinyl azobenzenes and in the studies of their photophysical properties.[5]

[4] a) S. Wada, M. Urano, H. Suzuki, *J. Org. Chem.* **2002**, *67*, 8254-8257. b) E. Leyva, M. S. Plats, G. Persy, J. Wirz, *J. Am. Chem. Soc.*, **1986**, *108*, 3783-3790.

[5] (a) M. C. Carreño, G. Fernández Mudarra, E. Merino, M. Ribagorda, *J. Org. Chem*. **2004**,*69*, 3413-3416 (b) M. C. Carreño, I. García, M. Ribagorda, E. Merino, S. Pieraccini, G. P. Spada, *Org. Lett*. **2005**, *7*, 2869-2872.

With the aim to cross the lines of research of the groups working at the *Università di Messina* and at the *Universidad Autónoma de Madrid*, the synthesis of an OPE system with an azobenzene core was envisaged, in order to study the photo-physical properties of the resulting products, as well as to study the photo-isomerization *cis-trans* of these new products.With this idea in mind, we tried to insert an azobenzene *core* in a OPE system. The synthesis was planed on the retrosynthesis shown in Figure 4.

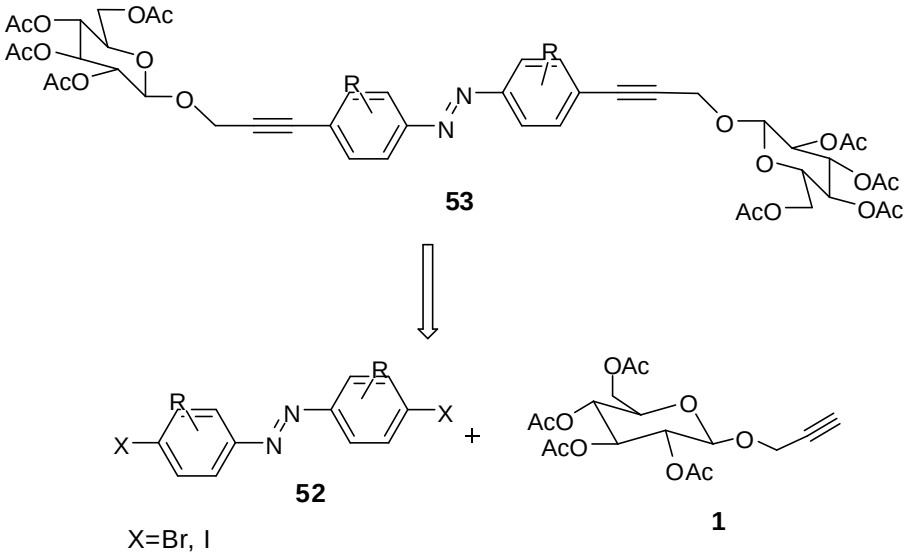


Figure 4

Thus, starting from an azobenzene having two aromatic halogenated moieties, the introduction of two phenylenenethynylene acetylenic moieties through a Sonogashira coupling could lead, directly to the target structure **53**. coupling.

As seen before (figure 3), the most frequently used method for the synthesis of the azo-compound is the azoic coupling between an aryldiazonium salt and an activated aromatic compounds. This reaction is based on an initial reaction of a primary aromatic amine with sodium nitrite under acidic conditions at low temperature giving an intermediate diazonium salt,

which is then reacted with an aromatic nucleophilic compound. Generally, the coupling is very fast and leads to the formation of azobenzene with overall good yields.

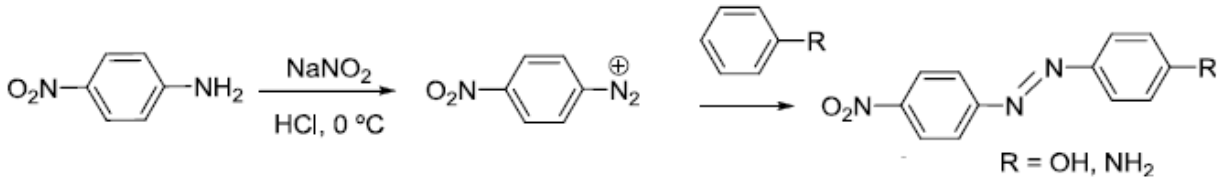
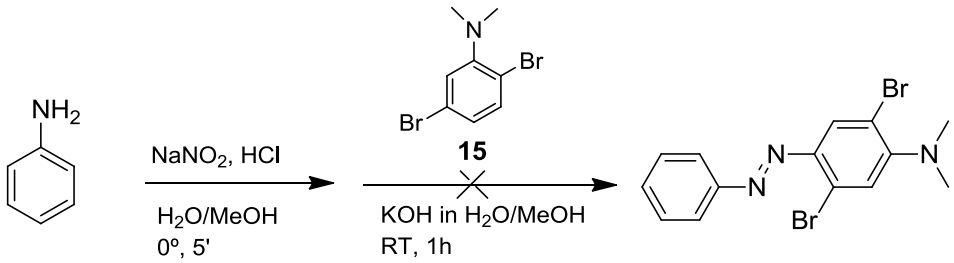


Figure 4

The first attempt to obtain an aza-core was performed using the azoic coupling on the previously synthesized compound **15** (Chapter 2), already containing two aromatic arms able to react in the Sonogashira reaction.

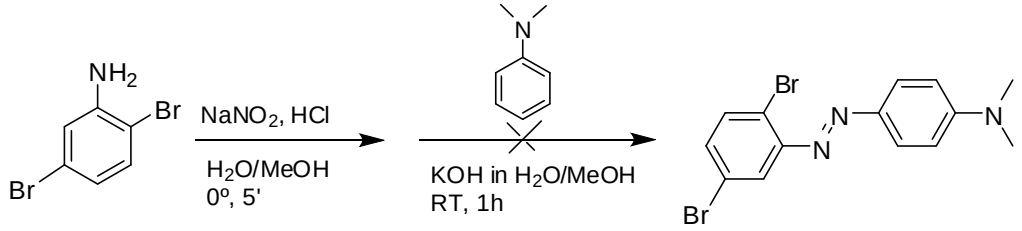
The *in situ* formation of diazobenzene was attempted in controlled conditions, followed by the slow addition of a solution of compound **15** and KOH (Scheme 1).



Scheme 1

The formation of the desired azobenzene wasn't observed by TLC, and this negative result was confirmed by ^{1}H-NMR analysis: in fact, only the signals of starting product **15** were present in the final spectrum.

A second attempt was performed utilizing the same method shown in scheme 1 but changing the starting products: also in this case, the reaction between commercial 2,5-dibromoaniline and dimethylaniline was totally uncessfull.



Scheme 2

An alternative synthetic method was found after a careful research in the literature: a copper-catalyzed approach (Figure 5) to the synthesis of symmetric and asymmetric aromatic azo-compounds.[6]

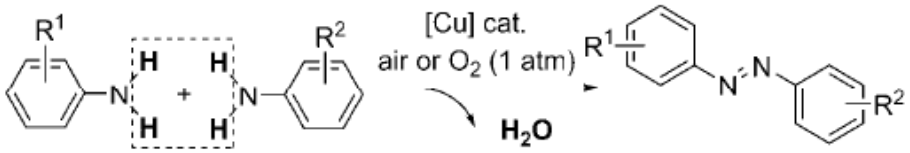
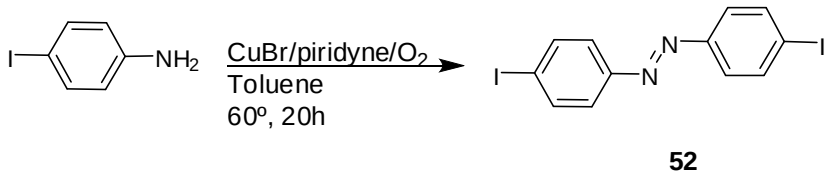


Figure 5

This method was applied to the synthesis of a new *core*: azobenzene **52**, containing two –I groups at the aromatic rings, fundamental to further react in Sonogashira conditions and to form the two new C≡C bonds.

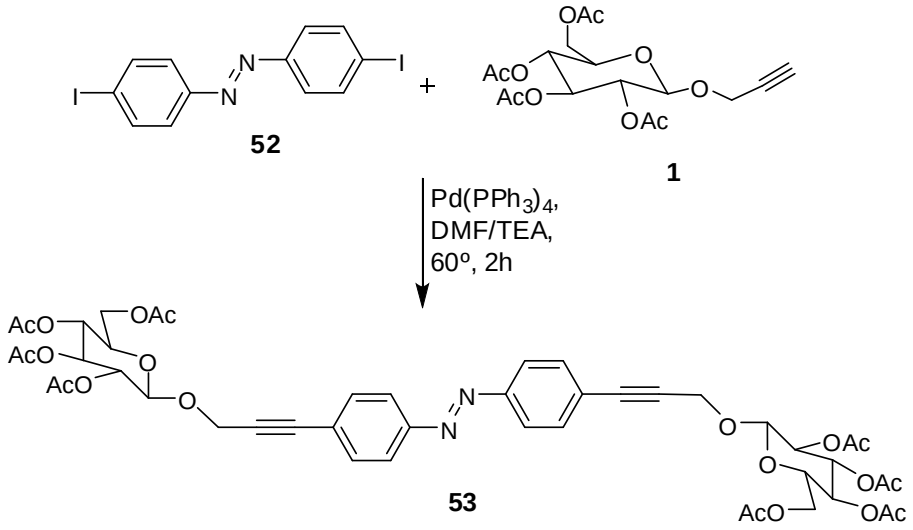


52

Scheme 3

[6] Zhang, C.; Jiao, N.; *Angew. Chem. Int. Ed*. **2010**, 49, 6174 –6177

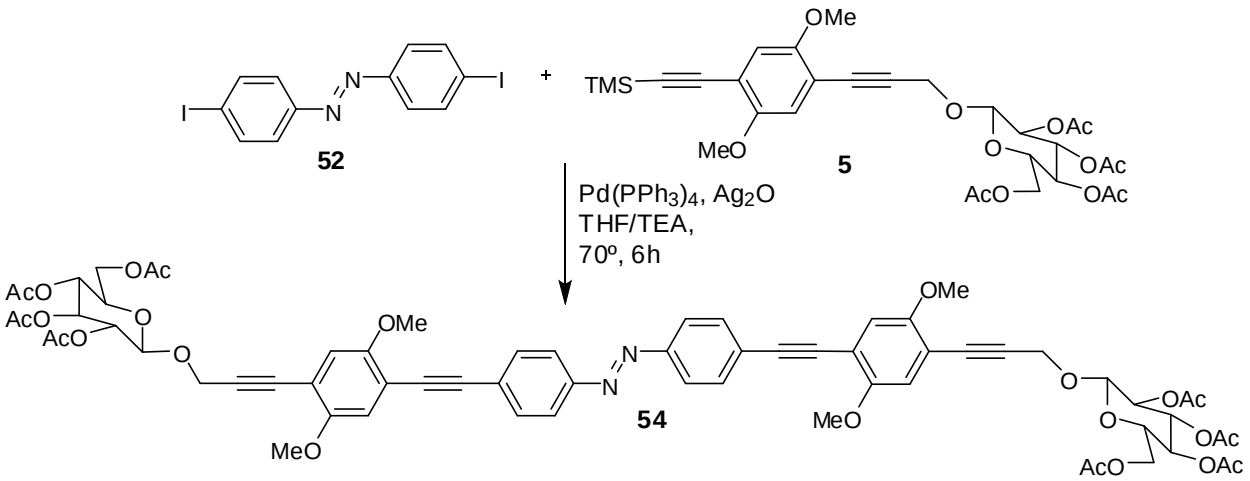
Compound **52** was synthesized starting from commercial *p*-iodoaniline, in presence of CuBr and pyridine mixed in toluene under O_2 (1 atm). The reaction mixture was stirred vigorously at 60°C for 20 h to afford compound **52** in quantitative yield.(Scheme 3)



Scheme 4

To test the reactivity of **52** in our copper-free Sonogashira conditions it was reacted at 60°C with peracetylated propargyl glucoside **1** (in 1:2 molecular ratio) in dry DMF and Et_3N, in the presence of $Pd(PPh_3)_4$. After 2 hours the reaction was complete and led to the formation of **50** as a red powder after chromatographic separation. The reaction occurred in good yield. Compound **53** was identified by NMR as the most stable *trans* (E) stereoisomer..

Having shown that azocompound **52** could react in cross-coupling conditions, we attempted the synthesis of **54**. Recation of an equimolecular amount of azobenzene **52** and glucoside derivate **5** (Chapter 2), in a mixture 2:1 DMF/THF, in presence of Ag_2O and a catalytic amount of $Pd(PPh_3)_4$ (Scheme 5) was carried carried out to give the azo-OPE **54**, that could be obtained as a brilliant orange powder by chromatography with a 67% isolated yield.



Scheme 5

4.2 Photoisomerization Studies

The absorption spectra of **53** and **54** in MeOH are reported in Figure 6. In the case of **53** the absorption profile is dominated by an intense band appearing at λ_{max} = 360 nm, which corresponds to the azobenzene $\pi \rightarrow \pi^*$ absorption. A less intense band is observed at λ_{max} = 450 nm, which is assigned to a $n \rightarrow \pi^*$ transition. In compound **54,** the former contribution can be recognized superimposed on the much stronger OPE band at 416 nm, while the latter is completely covered by a tail on the visible region.

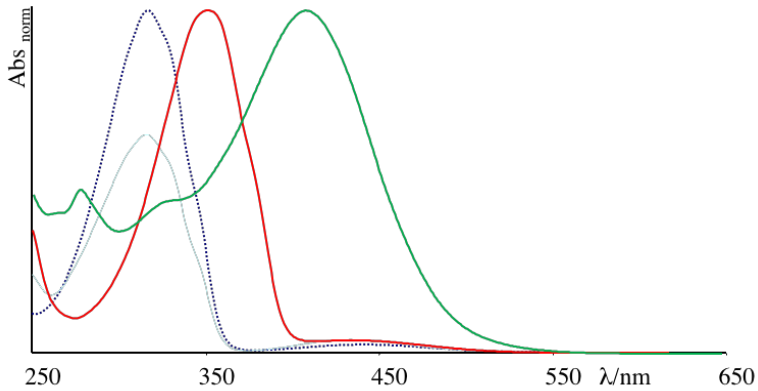


Figure 6. Normalized absorption spectra of Azobenzene, **53**, **54** in MeOH.

Preliminary experiments have been performed to investigate the photoisomerization ability of these new species.

Irradiation at 360 nm of a methanol solution of **53** and **54** at 298 K leads to strong spectral changes, as expected for the trans - cis photoisomerization of the azobenzene moieties, shown in Figures 7 and 8.

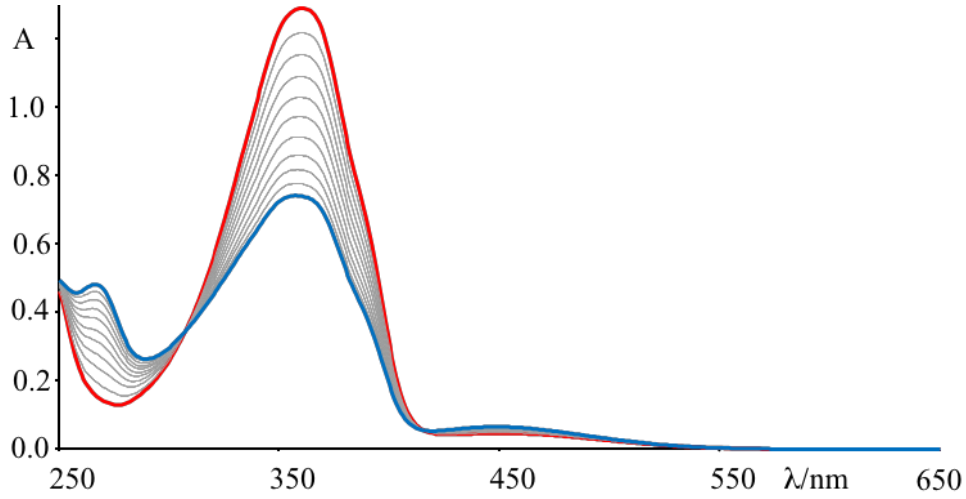


Figure 7. Trans→Cis photo-isomerization of **53** in methanol at room temperature λ_{exc}=360 nm

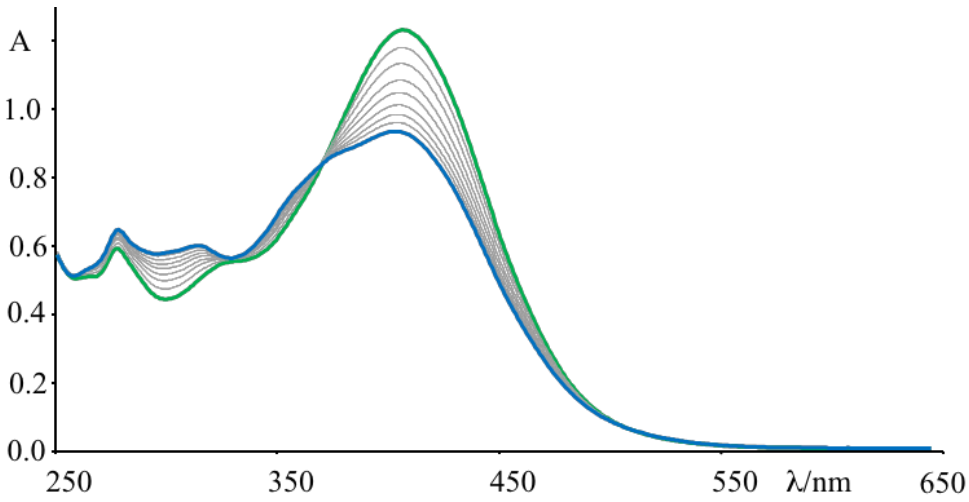


Figure 8. Trans→Cis photo-isomerization of **54** in methanol at room temperature λ_{exc}=410 nm.

In both cases, on continued irradiation, a photostationary state is reached. The initial trans-species can be completely recovered by keeping the solution in the dark. The thermal cis - trans reaction at 298 K is quite slow ($t_{1/2}$ = 32 h), so that the cis-species could be deeply studied.

The trans - cis photoisomerization quantum yields (upon irradiation at the absorbtion maxima) are very similar for the two investigated species in MeOH (ϕ= 0.08 @360 nm for **53** and ϕ= 0.09 @360 nm for **54**) but smaller to those of the model azobenzene compound (ϕ= 0.13 in MeOH λ_{exc}=365 nm). The wavelength dependence of this behaviour is still under investigation in more details but the lowering of isomerization quantum yields is typical of sterically hindered azobenzenes.

Conclusions

Chapter 5

The synthetic design and optimization of the carbohydrate-functionalized oligo(phenyleneethynylene)s developed during this PhD thesis, allowed to withdraw the following conclusions:

1. A strategy based on the exploitation of Pd(0) catalyzed cross-coupling reactions between alkynes and halogen substituted aryl derivatives has allowed the synthesis of a new family of OPEs in a convergent manner.

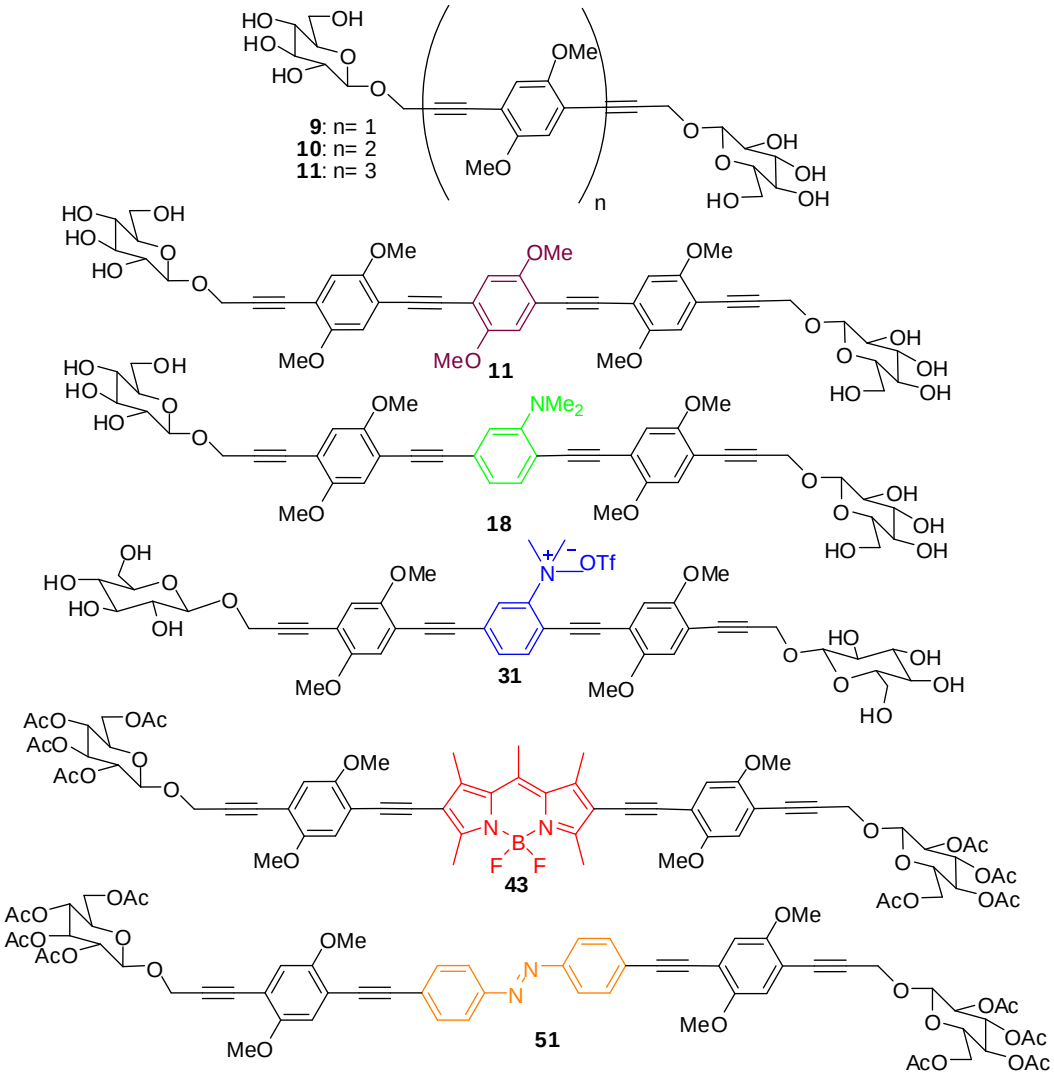


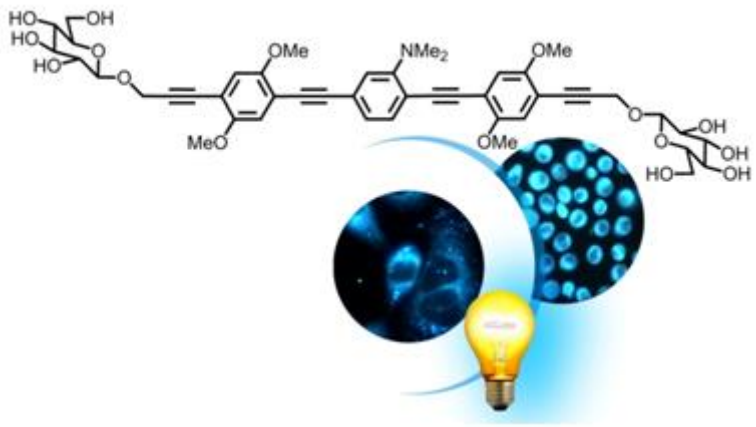
Figure 1

A peracetylated carbohydrate moiety (glucose or galactose) is incorporated as a propargyl glycoside which is used in one of these couplings. The great applicability of the methodology was demonstrated through the synthesis of phenyleneethynylene glycosides of different lengths and having different substituents at the central *core* such as *p*-dimethoxy phenyl, benzene, N,N-dimethyl anilines and their ammonium salts, BODIPY and azobenzenes.

In Figure 1 some of the compounds synthesized with different substituents: OMe, NMe_2, $^{+}NMe_3$ (**11**, **18** and **31**) on aromatic central ring and the hybrid OPEs with BODIPY and azobenzene central unities (**43** and **51**) are shown.

2. The photophysical properties as wavelength of absorption and emission and quantum yieldswere studied. The synthesized OPEs adsorb and emit at blue-green wavelengths (350-480nm).Quantum yield (Φ), for the OPEs with three phenylenethynylene units, are higher than 0.57.Generation of singlet oxygen was demonstrated to be efficient for compounds **18** and **28**.

Their biocompatibility *in vitro* was demonstrated by their high luminescence at low concentrations (1µm) and internalization in the



cells.

Figure 2

The amino derived compounds **18** and **28** were shown to be active as photosensitizers in Photo Dinamic Therapy when irradiated with UVA light , due to the generation of singlet oxygen which provokes cell death.

3. An initial study of photochromic properties of OPE azobenzene derivatives **50** and **51** has been carried out to evaluate their photoisomerization ability. Using UV/vis spectroscopy, the photoisomerization was shown to occur. Other techniques (mainly NMR and CD) should be applied to investigate if these carbohydrate substituted Azobenzene OPEs fill the criteria to be used as enantiopure, water soluble molecular switches.

5.1 Publications:

Part of the work developed in this Ph. D. work has been published:

Barattucci, A.; Deni, E.; Bonaccorsi, P.; Ceraolo, M. G.; Papalia, T.; Santoro, A.; Sciortino, M. T.; Puntoriero, F., *J. Org. Chem.* **2014**, 79, 5113.

Barattucci, A. Aversa, M. C.; Deni, E.;Papalia, T.; Bonaccorsi, P.; *Helvetica Chimica Acta* **2014**, 97, 1237.

Deni, E.; Zamarrón, A.; Bonaccorsi, P.; Carreño, M.C.; Juarranz, A.; Puntoriero, F.; Sciortino, M.T.; Ribagorda, M.; Barattucci,A., *Eur. J. Med. Chem.*, **2016**, (submitted).

Experimental Section

Chapter 6

6.1 General Synthetic Methods.

Solvents were purified according to standard procedures. All of the reactions were monitored by TLC on commercially available precoated plates (silica gel 60 F254), and the products were visualized with vanillin [1 g dissolved in MeOH (60 mL) and conc. H_2SO_4 (0.6 mL)] and UV lamp. Silica gel 60 was used for column chromatography. Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a Varian 500 spectrometer (at 500 MHz for ^1H; and 125 MHz for ^{13}C) or a AV-300 Bruker (at 300 MHz for ^1H; and 75 MHz for ^{13}C) using $CDCl_3$ as solvent, unless differently stated. Chemical shifts are given in parts per million (ppm) (δ relative to residual solvent peak for ^1H and ^{13}C: δ 7.26 and 77.0 ppm respectively), coupling constants (*J*) are given in hertz; the attributions are supported by heteronuclear single-quantum coherence (HSQC), Heteronuclear Multiple Bond Correlation (HMBC) and correlation spectroscopy (COSY) experiments, and given in accordance with the numeration indicated in the designed structures. Mass spectra (m/z) and HRMS were recorded under the conditions of electron impact (EI) and electrospray (ES) in a Waters VG Autospect spectrometer. Melting points were obtained in open capillary tubes and are uncorrected.

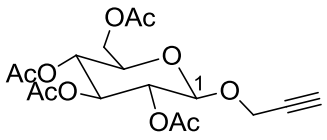
qNMR analysis protocol: 530 μL aliquots of a *dmso-d6* solution of a known concentration of **18** or **28** were transferred into a 5 mm WILMAD NMR tube into which a stem coaxial tube (WILMAD, WGS-5BL, 50 mm L × 2 mm stem o.d.), loaded with 60 μL of a 29.11 mM *dmso-d6* solution of maleic acid TraceCERT® (Sigma-Aldrich) was subsequently inserted. Accurate integration of the CH resonance of maleic acid (δ = 6.28 ppm) *vs* significant aliphatic peaks of **1** (5.14, 4.67 and 4.33 ppm) and **2** (5.14, 4.64 and 4.31ppm) provided the effective compound concentration, then compared with the calculated one. 90° pulse excitation, 15 s recycle delay,

and 64 transients.

A general procedure for **deacetilation** of compounds **3**, **7**, **8**, **12**, **17**, **27**, **29**, **30**, **35**, **37** is given: **General Procedure A.** The starting product (0.2mmol) was dissolved in of THF-MeOH (1:1, 40mL). To this mixture a large excess of aqueous ammonia (12mL) was added and the reaction was then maintained under continuous stirring at RT overnight, until the disappearance of the starting product by TLC. Solvents were removed under reduced pressure and the undesired acetamide was eliminated by a series of MeOH washings of the obtained solid.

2-Propyn-1-yl-β-D-glucopyranoside 2,3,4,6-tetraacetate **1**[1]

To a solution of 1,2,3,4,6-penta-*O*-acetil-β-D-glucopyranose (5.0g, 12.55 mmol) in 36 mL of dry CH_2Cl_2, at 0°C and under Argon, 1.48 mL of propargyl alchol (25.10 mmol, d = 0.958 g/mL), 4.59 mL di trimethylsilyltriflate (25.10 mmol, d=1.228 g/mL) and molecular sieves 4Å were added. The mixture was heated to RT and maintained under continuous stirring until the disappearance of **1** by TLC (1 hour). The reaction was quenched by adding $NaHCO_3$ (40 mL) and the organic phase washed with water (2 x 30 mL). The organic phases were dried over anhydrous Na_2SO_4, the solvent evaporated under reduced pressure. Column chromatography was performed with hexane/EtOAc 70:30 as eluant, and compound **1** was obtained as a white solid (3.4 g, 70%). TLC: *Rf* = 0.50 (hexane/EtOAc 70:30).



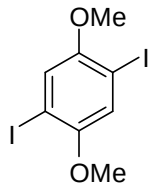
1

1,4-Diiodo-2,5-dimethoxybenzene **2.**[2]

[1] Giovenzana, G. B.; Lay, L.; Monti, D.; Palmisano, G.; Panza, L. *Tetrahedron* **1999**, *55*, 14123-14136.

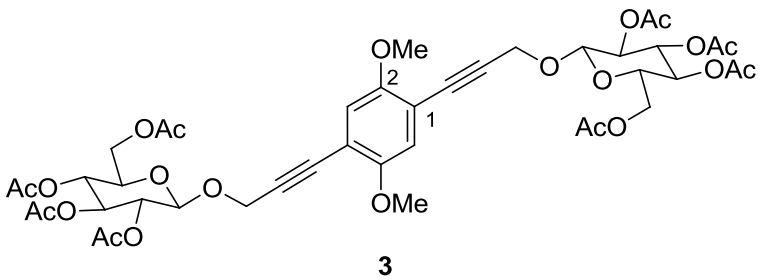
[2] Yi, C.; Blum, C.; Lehamann, M.; Keller, S.; Liu, S. X.; Frei, G.; Neels, A.; Hauser, J.; Schürch, S.; Decurtins, S. *J. Org. Chem.* **2010**, *75*, 3350-3357.

To a solution of H_5IO_6 (0.73 g, 3.44 mmol) in 5 mL of CH_3OH, I_2 (1.60 g, 15.10 mmol) was added; after ten minutes under vigorous stirring at RT commercial 1,4-dimethoxybenzene (0.675 g, 2.78 mmol) was added. The mixture was to reflux and maintained under continuous stirring for 4h. The reaction was quenched by adding a solution of $Na_2S_2O_5$ 10%wt (5 mL) and extracted with dichloromethane (3 x 10 mL). The organic phases were washed with brine and then dried over anhydrous Na_2SO_4. The solvent evaporated under reduced pressure to give **2** as a pale yellow solid (1.80 g, 4.63mmol, 95%).



2

Compound 3. 2-propyn-1-yl-β-D-glucopyranoside 2,3,4,6-tetraacetate **1** (1.00 g, 2.59 mmol), 1,4-diiodo-2,5-dimethoxybenzene **2** (0.48 g, 1.23 mmol), $Pd(PPh_3)_4$ (0.17 g, 0.15 mmol) were dissolved in dry DMF (10 mL). To this mixture Et_3N (10 mL) was slowly added. The mixture, under Ar atmosphere, was heated at 65 °C and maintained under continuous stirring for 2 h until the disappearance of **2** by TLC. Column chromatography was performed with hexane/EtOAc 65:35 as eluant, and compound **3** was obtained as a white solid (0.80 g, 0.88 mmol, 72%).

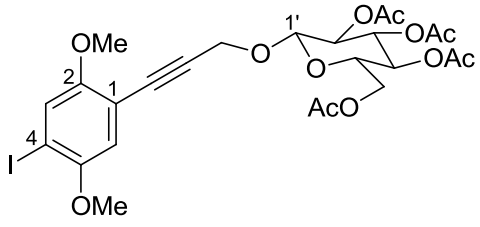


3

TLC: R_f 0.55 (hexane/EtOAc 40:60). Mp 178-180 °C. ^{1}H NMR: δ 6.92 (s, 2H, H-3,6), 5.27 (t, $J_{2',3'} = J_{3',4'} = 9.3$, 2H, 2 x H-3'), 5.12 (t, $J_{3',4'} = J_{4',5'} = 9.3$, 2H, 2 x H-4'), 5.05 (dd, $J_{1',2'} = 8.3$, $J_{2',3'} = 9.3$, 2H, 2 x H-2'), 4.90 (d, $J_{1',2'} = 8.3$, 2H, 2 x H-1'), 4.64 (s, 4H, 2 x $CH_2C\equiv$), 4.28 and 4.17

(split AB system, $J_{5',6'A}$ = 5.6, $J_{5',6'B}$ = 2.4, $J_{6'A,6'B}$ = 12.2, 4H, 2 x H_2-6'), 3.85 (s, 6H, 2 x OCH_3), 3.76 (ddd, $J_{4',5'}$ = 9.3, $J_{5',6'A}$ = 5.6, $J_{5',6'B}$ = 2.4, 2H, 2 x H-5'), 2.07, 2.04, 2.02, and 2.00 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.7, 170.3, 169.5, and 169.4 (8 x CO), 154.0 (C-2,5), 115.7 (C-3,6), 112.8 (C-1,4), 98.3 (2 x C-1'), 89.2 and 83.2 (2 x C≡C), 72.8 (2 x C-3'), 71.9 (2 x C-5'), 71.1 (2 x C-2'), 68.3 (2 x C-4'), 61.8 (2 x C-6'), 57.0 (2 x $\underline{C}H_2C\equiv$), 56.4 (2 x OCH_3), 20.7 and 20.6 (8 x $\underline{C}H_3CO$). Anal. Calcd for $C_{42}H_{50}O_{22}$ (906.83): C, 55.63; H, 5.56. Found: C, 55.49; H, 5.58.

Compound 4. 2-propyn-1-yl β-D-glucopyranoside 2,3,4,6-tetraacetate **1** (1.42 g, 3.67 mmol), 1,4-diiodo-2,5-dimethoxybenzene **2** (3.00 g, 7.69 mmol) and $Pd(PPh_3)_4$ (0.50 g, 0.43 mmol) were dissolved in dry DMF (30 mL). To the mixture Et_3N (30 mL) was slowly added. The mixture was heated at 60 °C and maintained under continuous stirring and under Ar atmosphere for 3h, until the disappearance of **1** by TLC. Column chromatography was performed with hexane/EtOAc 70:30 as eluant, and compound **4** was obtained as a white solid (1.90 g, 2.93 mmol, 80%).

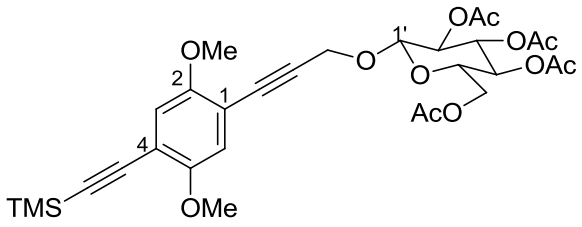


4

TLC: R_f 0.64 (hexane/EtOAc 40:60). Mp 65-67 °C. ^{1}H NMR: δ 7.29 (s, 1H, H-3), 6.84 (s, 1H, H-6), 5.25 (t, $J_{2',3'}$ = $J_{3',4'}$ = 9.3, 1H, H-3'), 5.11 (t, $J_{3',4'}$ = $J_{4',5'}$ = 9.3, 1H, H-4'), 5.03 (dd, $J_{1',2'}$ = 7.7, $J_{2',3'}$ = 9.3, 1H, H-2'), 4.88 (d, $J_{1',2'}$ = 7.7, 1H, H-1'), 4.61 (s, 2H, $CH_2C\equiv$), 4.27 and 4.15 (split AB system, $J_{5',6'A}$ = 4.4, $J_{5',6'B}$ = 2.5, $J_{6'A,6'B}$ = 12.2, 2H, H_2-6'), 3.84 and 3.83 (two s, 6H, 2 x OCH_3), 3.75 (ddd, $J_{4',5'}$ = 9.3, $J_{5',6'A}$ = 4.4, $J_{5',6'B}$ = 2.5, 1H, H-5'), 2.07, 2.03, 2.02, and 2.00 (four s, 12H, 4 x CH_3CO). ^{13}C NMR: δ 170.7, 170.3, and 169.4 (4 x CO), 154.8 (C-2), 152.3 (C-5), 122.3 (C-3),

115.1 (C-6), 111.9 (C-1), 98.3 (C-1'), 88.4 (C-4), 87.3 and 85.4 (C≡C), 72.8 (C-3'), 71.9 (C-5'), 71.1 (C-2'), 68.4 (C-4'), 61.8 (C-6'), 57.2, 57.0, and 56.5 (2 x OCH_3 and $\underline{C}H_2C\equiv$), 20.7 and 20.6 (4 x $\underline{C}H_3CO$). Anal. Calcd for $C_{25}H_{29}IO_{12}$ (648.40): C, 46.31; H, 4.51. Found: C, 46.44; H, 4.50.

Compound 5. To a solution of compound **4** (1.00 g, 1.54 mmol) and $Pd(PPh_3)_4$ (0.18 g, 0.16 mmol) in dry DMF (7 mL), commercial Ethynyltrimethylsilane (0.65 mL, 4.62 mmol) and dry Et_3N (7 mL) were slowly added. The mixture, under Ar atmosphere, was heated at 65 °C and maintained under continuous stirring for 2h, until the disappearance of **4** by TLC. Column chromatography was performed with hexane/EtOAc 80:20 as eluant, and compound **5** was obtained as a white solid (0.81 g, 1.31 mmol, 85%).

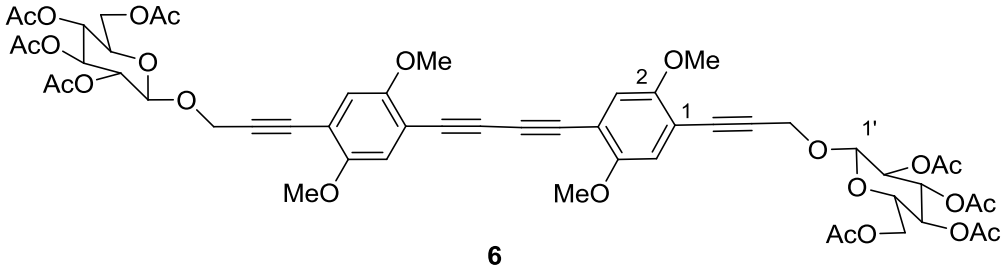


5

TLC: R_f 0.78 (hexane/EtOAc 40:60). Mp 59-61 °C. ^{1}H NMR: δ 6.95 and 6.89 (two s, 2H, H-3,6), 5.25 (t, $J_{2',3'} = J_{3',4'} = 9.4$, 1H, H-3'), 5.10 (t, $J_{3',4'} = J_{4',5'} = 9.4$, 1H, H-4'), 5.03 (dd, $J_{1',2'} = 7.7$, $J_{2',3'} = 9.4$, 1H, H-2'), 4.88 (d, $J_{1',2'} = 7.7$, 1H, H-1'), 4.62 (s, 2H, $CH_2C\equiv$), 4.28 and 4.15 (split AB system, $J_{5',6'A} = 4.7$, $J_{5',6'B} = 2.4$, $J_{6'A,6'B} = 12.2$, 2H, H_2-6'), 3.85 and 3.84 (two s, 6H, 2 x OCH_3), 3.74 (ddd, $J_{4',5'} = 10.0$, $J_{5',6'A} = 4.7$, $J_{5',6'B} = 2.5$, 1H, H-5'), 2.07, 2.04, 2.03, and 2.01 (four s, 12H, 4 x CH_3CO), 0.27 [s, 9H, $Si(CH_3)_3$]. ^{13}C NMR: δ 170.7, 170.3, 169.4, and 169.3 (4 x CO), 154.2 and 153.9 (C-2,5), 116.1 and 115.8 (C-3,6), 113.7 and 112.4 (C-1,4), 100.7 and 100.6 (C≡CSi), 98.2 (C-1'), 89.0 and 83.4 ($CH_2\underline{C}\equiv\underline{C}$), 72.8 (C-3'), 71.9 (C-5'), 71.1 (C-2'), 68.3 (C-4'), 61.8 (C-6'), 57.0 ($\underline{C}H_2C\equiv$), 56.5 and 56.3 (2 x OCH_3), 20.7, 20.6, and 20.5 (4 x $\underline{C}H_3CO$), - 0.05 [$Si(CH_3)_3$]. Anal. Calcd for $C_{30}H_{38}O_{12}Si$ (618.70): C, 58.24; H, 6.19. Found: C, 58.30; H, 6.20

C**ompounds 6 and 7. C**ompounds **4** (0.50 g, 0.77 mmol) and **5** (0.48 g, 0.78 mmol), $Pd(PPh_3)_4$ (0.13 g, 0.11 mmol), and Ag_2O (0.18 g, 0.78 mmol) were suspended in a mixture (1:2) of dry DMF (10 mL) and THF (5 mL). The mixture was heated at 70 °C and left under Ar atmosphere and continuous stirring until completation of the reaction (5 hours). After filtration over Celite, the solvents were removed under reduced pressure, and the obtained reaction crude was subjected to silica gel column chromatography using hexane/EtOAc 50:50 as eluant, to obtain first compound **6** as a yellow low-melting solid (42 mg, 0.04 mmol, 5%) and then compound **7** as a yellow solid (0.44 g, 0.41 mmol, 53%).

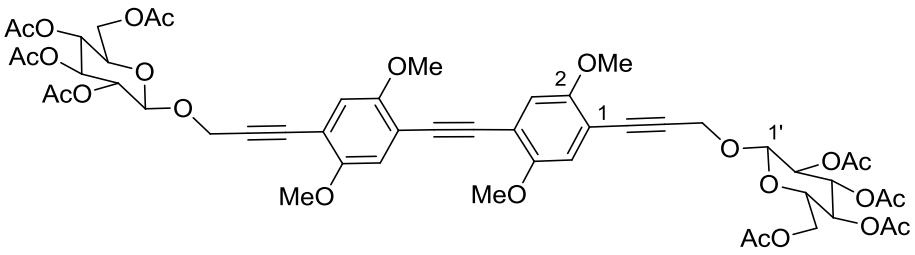
Compound 6.



6

TLC: R_f 0.40 (hexane/EtOAc 30:70). ^{1}H NMR: δ 6.98 and 6.92 (two s, 4H, 2 x H-3,6), 5.26 (t, $J_{2',3'} = J_{3',4'} = 9.3$, 2H, 2 x H-3'), 5.12 (t, $J_{3',4'} = J_{4',5'} = 9.3$, 2H, 2 x H-4'), 5.04 (t, $J_{1',2'} = J_{2',3'} = 9.3$, 2H, 2 x H-2'), 4.90 (d, $J_{1',2'} = 9.3$, 2H, 2 x H-1'), 4.64 (s, 4H, 2 x $CH_2C{\equiv}$), 4.28 and 4.17 (split AB system, $J_{5',6'A} = 4.4$, $J_{5',6'B} = 1.9$, $J_{6'A,6'B} = 12.2$, 4H, 2 x H_2-6'), 3.86 and 3.84 (two s, 12H, 4 x OCH_3), 3.76 (ddd, $J_{4',5'} = 9.3$, $J_{5',6'A} = 4.4$, $J_{5',6'B} = 1.9$, 2H, 2 x H-5'), 2.08, 2.04, 2.03, and 2.01 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.9, 170.6, 169.7, and 169.6 (8 x CO), 155.6 and 154.2 (2 x C-2,5), 116.3 and 115.9 (2 x C-3,6), 113.6 and 112.6 (2 x C-1,4), 98.5 (2 x C-1'), 90.0, 83.5, 79.7, and 79.6 (4 x C≡C), 73.1 (2 x C-3'), 72.2 (2 x C-5'), 71.4 (2 x C-2'), 68.6 (2 x C-4'), 62.1 (2 x C-6'), 57.3 (2 x $\underline{C}H_2C{\equiv}$), 56.7 and 56.6 (4 x OCH_3), 20.9 and 20.8 (8 x $\underline{C}H_3CO$). Anal. Calcd for $C_{54}H_{58}O_{24}$ (1091.02): C, 59.45; H, 5.36. Found: C, 59.38; H, 5.34.

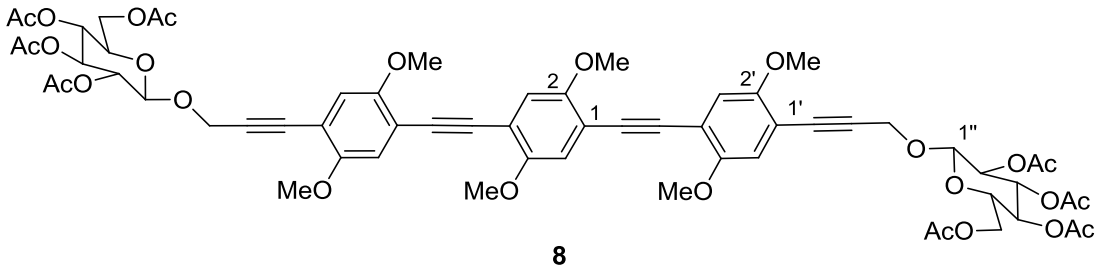
Compound 7.



7

TLC: R_f 0.35 (hexane/EtOAc 30:70). Mp 80-81 °C. ^{1}H NMR: δ 7.04 and 6.94 (two s, 4H, 2 x H-3,6), 5.27 (t, $J_{2',3'} = J_{3',4'} = 9.3$, 2H, 2 x H-3'), 5.12 (t, $J_{3',4'} = J_{4',5'} = 9.3$, 2H, 2 x H-4'), 5.05 (dd, $J_{1',2'} = 8.3$, $J_{2',3'} = 9.3$, 2H, 2 x H-2'), 4.92 (d, $J_{1',2'} = 8.3$, 2H, 2 x H-1'), 4.65 (s, 4H, 2 x $CH_2C{\equiv}$), 4.28 and 4.17 (split AB system, $J_{5',6'A} = 4.4$, $J_{5',6'B} = 2.4$, $J_{6'A,6'B} = 12.3$, 4H, 2 x H_2-6'), 3.90 and 3.88 (two s, 12H, 4 x OCH_3), 3.77 (ddd, $J_{4',5'} = 9.3$, $J_{5',6'A} = 4.4$, $J_{5',6'B} = 2.4$, 2H, 2 x H-5'), 2.08, 2.05, 2.03, and 2.01 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.7, 170.3, 169.4, and 169.3 (8 x CO), 154.1 and 153.9 (2 x C-2,5), 115.7 and 115.5 (2 x C-3,6), 113.8 and 112.4 (2 x C-1,4), 98.3 (2 x C-1'), 91.2, 89.1, and 83.4 (3 x C≡C), 72.8 (2 x C-3'), 71.9 (2 x C-5'), 71.1 (2 x C-2'), 68.4 (2 x C-4'), 61.8 (2 x C-6'), 57.0 (2 x $\underline{C}H_2C{\equiv}$), 56.5 and 56.3 (4 x OCH_3), 20.7 and 20.6 (8 x $\underline{C}H_3CO$). Anal. Calcd for $C_{52}H_{58}O_{24}$ (1067.00): C, 58.53; H, 5.48. Found: C, 58.65; H, 5.47.

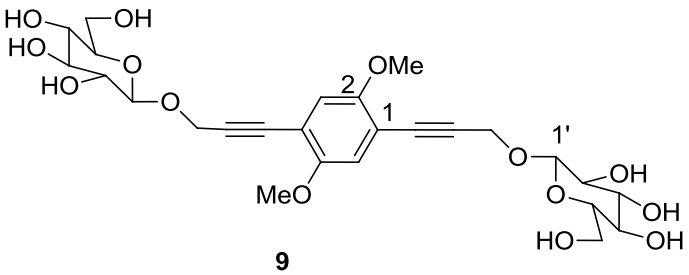
Compound 8. Compounds **2** (0.50 g, 1.28 mmol), **5** (1.58 g, 2.56 mmol), $Pd(PPh_3)_4$ (0.22 g, 0.19 mmol) and Ag_2O (0.59 g, 2.55 mmol) were suspended in a mixture (1:2) of dry DMF (10 mL) and dry THF (5 mL)**.** The mixture was heated at 70 °C for 6h and maintained under Ar atmosphere and continuous stirring until the disappearance of the starting products **2** and **5** by TLC (hexane/EtOAc 3:7). After filtration over Celite, the solvents were removed under reduced pressure, and on the reaction crude column chromatography was performed with hexane/EtOAc 50:50 as eluant, and compound **8** was obtained as a yellow solid (1.04 g, 0.85 mmol, 66%).



8

TLC: R_f 0.47 (hexane/EtOAc 40:60). Mp 169-171 °C. ^{1}H NMR: δ 7.06, 7.05, and 6.94 (three s, 6H, 3 x H-3,6), 5.27 (t, $J_{2',3'} = J_{3',4'} = 9.3$, 2H, 2 x H-3'), 5.12 (t, $J_{3',4'} = J_{4',5'} = 9.3$, 2H, 2 x H-4'), 5.05 (dd, $J_{1',2'} = 7.8$, $J_{2',3'} = 9.3$, 2H, 2 x H-2'), 4.91 (d, $J_{1',2'} = 7.8$, 2H, 2 x H-1'), 4.65 (s, 4H, 2 x $CH_2C\equiv$), 4.28 and 4.17 (split AB system, $J_{5',6'A} = 4.9$, $J_{5',6'B} = 2.4$, $J_{6'A,6'B} = 12.3$, 4H, 2 x H_2-6'), 3.92, 3.90, and 3.88 (three s, 18H, 6 x OCH_3), 3.77 (ddd, $J_{4',5'} = 9.3$, $J_{5',6'A} = 4.9$, $J_{5',6'B} = 2.4$, 2H, 2 x H-5'), 2.08, 2.05, 2.03, and 2.01 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.7, 170.3, 169.5, and 169.4 (8 x CO), 154.1, 153.9, and 153.8 (3 x C-2,5), 115.7 and 115.5 (3 x C-3,6), 113.8, 113.4, and 112.3 (3 x C-1,4), 98.3 (2 x C-1'), 91.5, 91.2, 89.1, and 83.5 (4 x C≡C), 72.8 (2 x C-3'), 71.9 (2 x C-5'), 71.1 (2 x C-2'), 68.3 (2 x C-4'), 61.8 (2 x C-6'), 57.1 (2 x $\underline{C}H_2C\equiv$), 56.6, 56.5 and 56.3(6 x OCH_3), 20.7 and 20.6 (8 x $\underline{C}H_3CO$). Anal. Calcd for $C_{62}H_{66}O_{26}$ (1227.17): C, 60.68; H, 5.42. Found: C, 60.80; H, 5.43.

Compound 9. It was obtained following **procedure A** starting from **3** (0.18 g), as a white solid (0.13 g, 0.19 mmol, 96%).

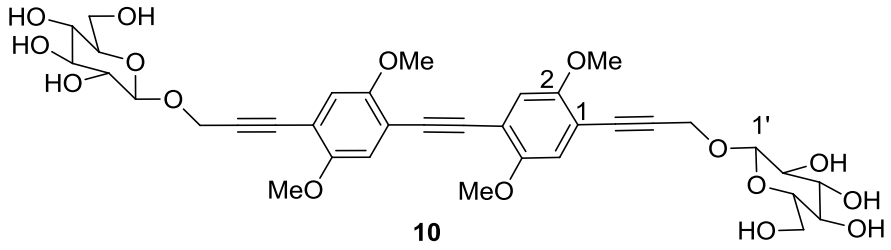


9

TLC: R_f 0.15 ($CHCl_3$/MeOH 80:20). Mp 230-233 °C. ^{1}H NMR (dmso-*d6*): δ 7.04 (s, 2H, H-3,6), 5.12 (d, $J_{vic} = 4.9$, 2H, 2 x OH), 4.96 (d, $J_{vic} = 4.9$, 2H, 2 x OH), 4.91 (d, $J_{vic} = 5.4$, 2H, 2 x OH)

, 4.65 and 4.52 (AB system, J_{gem} = 15.7, 4H, 2 x $CH_2C\equiv$), 4.53 (t, $J_{OH,6}$= 4.9, 2H, 2 x 6'-OH), 4.32 (d, $J_{1',2'}$ = 7.9, 2H, 2 x H-1'), 3.77 (s, 6H, 2 x OCH_3), 3.65 and 3.43 (split AB m, 4H, 2 x H_2-6'), 3.16 - 2.93 (m, 8H, 2 x H-2'-5'). ^{13}C NMR (dmso-*d6*): δ 153.6 (C-2,5), 115.8 (C-3,6), 112.2 (C-1,4), 101.1 (2 x C-1'), 91.1 and 81.9 (2 x C≡C), 77.0 and 76.7 (2 x C-3',5'), 73.3 (2 x C-2'), 70.0 (2 x C-4'), 61.2 (2 x C-6'), 56.1 (2 x OCH_3), 55.8 (2 x $\underline{C}H_2C\equiv$). Anal. Calcd for $C_{26}H_{34}O_{14}$ (570.54): C, 54.73; H, 6.01. Found: C, 54.79; H, 6.00.

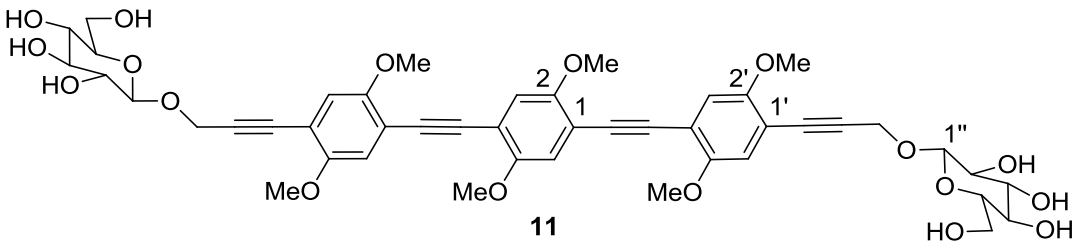
Compound 10. It was obtained following **procedure A** starting from **7** (0.23g), as a white solid (0.14 g, 0.19 mmol, 96%).



10

TLC: R_f 0.10 ($CHCl_3$/MeOH 80:20). Mp 206-207 °C. ^{1}H NMR (dmso-*d6*): δ 7.10 and 7.08 (two s, 4H, 2 x H-3,6), 5.14 (d, J_{vic} = 4.9, 2H, 2 x OH), 4.97 (d, J_{vic} = 4.9, 2H, 2 x OH), 4.91 (d, J_{vic} = 5.4, 2H, 2 x OH), 4.67 and 4.55 (AB system, J_{gem} = 15.6, 4H, 2 x $CH_2C\equiv$), 4.54 (t, $J_{OH,6}$= 4.9, 2H, 2 x 6'-OH), 4.34 (d, $J_{1',2'}$ = 7.8, 2H, 2 x H-1'), 3.81 and 3.80 (two s, 12H, 4 x OCH_3), 3.67 and 3.46 (split AB m, 4H, 2 x H_2-6'), 3.19 - 2.98 (m, 8H, 2 x H-2'-5'). ^{13}C NMR (dmso-*d6*): δ 153.7 and 153.2 (2 x C-2,5), 115.9 and 115.3 (2 x C-3,6), 112.8 and 112.2 (2 x C-1,4), 101.0 (2 x C-1'), 91.3, 91.1 and 81.9 (3 x C≡C), 77.0 and 76.7 (2 x C-3',5'), 73.3 (2 x C-2'), 70.1 (2 x C-4'), 61.2 (2 x C-6'), 56.3 and 56.1 (4 x OCH_3), 55.8 (2 x $\underline{C}H_2C\equiv$). Anal. Calcd for $C_{36}H_{42}O_{16}$ (730.71): C, 59.17; H, 5.79. Found: C, 59.14; H, 5.80.

Compound 11. It was obtained following **procedure A** starting from **8** (0.25g), as a yellow solid (0.17 g, 0.19 mmol, 96%).

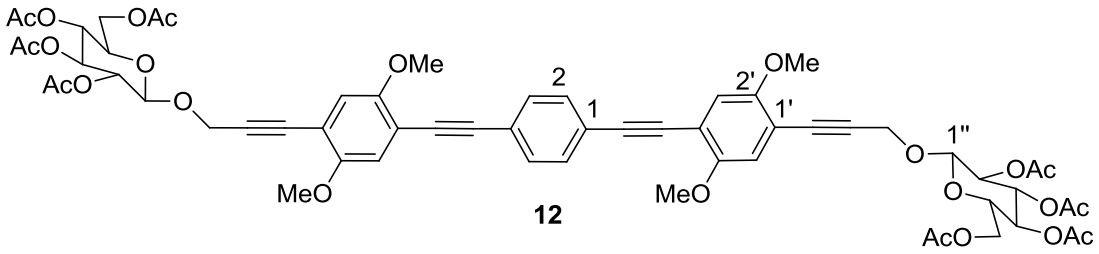


11

TLC: R_f 0.05 ($CHCl_3$/MeOH 80:20). Mp 248-250 °C. ^{1}H NMR (dmso-*d6*): δ 7.13, 7.12, and 7.09 (three s, 6H, 3 x H-3,6), 5.15 (d, J_{vic} = 5.4, 2H, 2 x OH), 4.98 (d, J_{vic} = 4.9, 2H, 2 x OH), 4.93 (d, J_{vic} = 5.4, 2H, 2 x OH), 4.68 and 4.55 (AB system, J_{gem} = 15.6, 4H, 2 x $CH_2C\equiv$), 4.55 (t, $J_{OH,6}$ = 5.5, 2H, 2 x 6'-OH), 4.34 (d, $J_{1',2'}$ = 7.9, 2H, 2 x H-1'), 3.84, 3.82, and 3.80 (three s, 18H, 6 x OCH_3), 3.69 and 3.45 (split AB m, 4H, 2 x H_2-6'), 3.19 - 2.98 (m, 8H, 2 x H-2'-5'). ^{13}C NMR (dmso-*d6*): δ 153.7, 152.4, and 153.3 (3 x C-2,5), 115.9,115.5, and 115.4 (3 x C-3,6), 112.8, 112.7 and 112.3 (3 x C-1,4), 101.1 (2 x C-1'), 91.3, 91.2 and 82.0 (4 x C≡C), 77.0 and 76.7 (2 x C-3',5'), 73.3 (2 x C-2'), 70.0 (2 x C-4'), 61.2 (2 x C-6'), 56.3, 56.2, and 56.1 (6 x OCH_3), 55.8 (2 x $\underline{C}H_2C\equiv$). Anal. Calcd for $C_{46}H_{50}O_{18}$ (890.88): C, 62.02; H, 5.66. Found: C, 62.05; H, 5.67.

Compounds 12 and 13. To a solution in dry DMF (12 mL) of **4** (1.98 g, 3.05 mmol, 2.1 equiv), commercial 1,4-diethynylbenzene (0.18 g, 1.43 mmol, 1 equiv) and $Pd(PPh_3)_4$ (0.20 g, 0.17 mmol, 0.12 equiv) dry Et_3N (12 mL) was slowly added. The obtained mixture was heated at 70°C and maintained under Ar atmosphere and continuous stirring until the disappearance of 1,4-diethynylbenzene by TLC (3 hours). Column chromatography was performed with hexane/EtOAc 60:40 as eluant, and a mixture of **12** and **13** was obtained**.** A second column chromatography (eluant toluene/acetonitrile 90:10) was necessary to obtain first **13** as a yellow solid (0.30 g, 0.23 mmol, 16%) and then **12** as a yellow solid (0.83 g, 0.71 mmol, 50%).

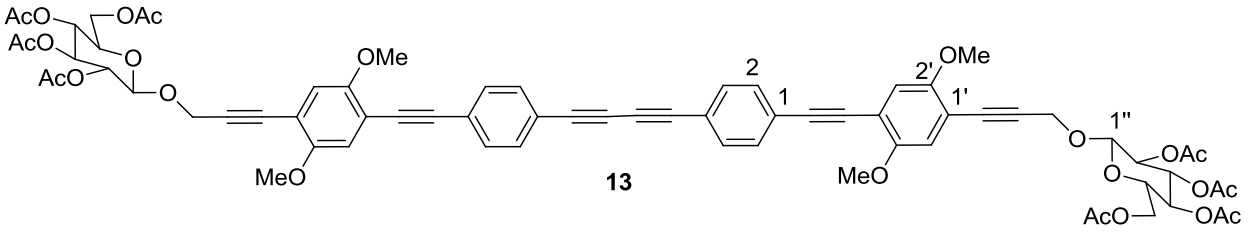
Compound 12.



12

TLC: R_f 0.59 (hexane/EtOAc 40:60). Mp 182-184 °C. ^{1}H NMR: δ 7.54 (s, 4H, H-2,3,5,6), 7.01 and 6.94 (two s, 4H, 2 x H-3',6'), 5.27 (t, $J_{2",3"} = J_{3",4"} = 9.3$, 2H, 2 x H-3"), 5.12 (t, $J_{3",4"} = J_{4",5"} = 9.3$, 2H, 2 x H-4"), 5.05 (dd, $J_{1",2"} = 8.3$, $J_{2",3"} = 9.3$, 2H, 2 x H-2"), 4.91 (d, $J_{1",2"} = 8.3$, 2H, 2 x H-1"), 4.65 (s, 4H, 2 x $CH_2C\equiv$), 4.28 and 4.17 (split AB system, $J_{5",6"A} = 4.9$, $J_{5",6"B} = 2.4$, $J_{6"A,6"B} = 12.2$, 4H, 2 x H_2-6"), 3.92 and 3.89 (two s, 12H, 4 x OCH_3), 3.76 (ddd, $J_{4",5"} = 9.3$, $J_{5",6"A} = 4.9$, $J_{5",6"B} = 2.4$, 2H, 2 x H-5"), 2.08, 2.05, 2.03, and 2.01 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.6, 170.3, 169.4, and 169.3 (8 x CO), 154.1 and 153.9 (2 x C-2',5'), 131.6 (C-2,3,5,6), 123.1 (C-1,4), 115.8 and 115.5 (2 x C-3',6'), 113.7 and 112.4 (2 x C-1',4'), 98.2 (2 x C-1"), 94.9, 89.1, 87.4, and 83.4 (4 x C≡C), 72.8 (2 x C-3"), 71.9 (2 x C-5"), 71.1 (2 x C-2"), 68.3 (2 x C-4"), 61.8 (2 x C-6"), 57.0 (2 x $\underline{C}H_2C\equiv$), 56.5 and 56.3 (4 x OCH_3), 20.7 and 20.6 (8 x $\underline{C}H_3CO$). Anal. Calcd for $C_{60}H_{62}O_{24}$ (1167.12): C, 61.75; H, 5.35. Found: C, 61.62; H, 5.36.

Compound 13.

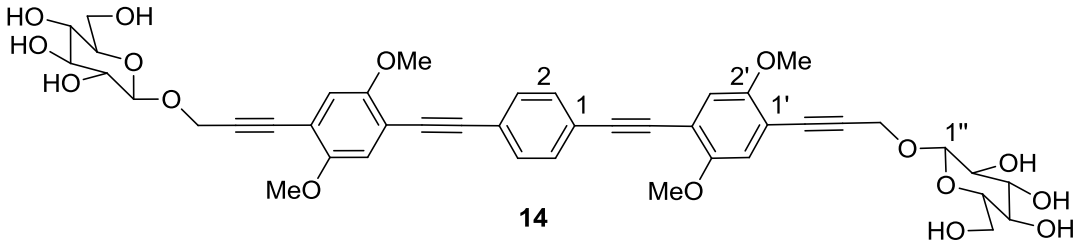


13

TLC: R_f 0.63 (hexane/EtOAc 40:60). Mp 109-111 °C. ^{1}H NMR: δ 7.52 (m, 8H, 2 x H-2,3,5,6), 7.01 and 6.95 (two s, 4H, 2 x H-3',6'), 5.27 (t, $J_{2",3"} = J_{3",4"} = 9.3$, 2H, 2 x H-3"), 5.12 (t, $J_{3",4"} = J_{4",5"} = 9.3$, 2H, 2 x H-4"), 5.05 (dd, $J_{1",2"} = 7.8$, $J_{2",3"} = 9.3$, 2H, 2 x H-2"), 4.91 (d, $J_{1",2"} = 7.8$, 2H, 2 x H-1"), 4.65 (s, 4H, 2 x $CH_2C\equiv$), 4.28 and 4.17 (split AB system, $J_{5",6"A} = 4.9$, $J_{5",6"B} = 2.4$, $J_{6"A,6"B} = 12.2$, 4H, 2 x H_2-6"), 3.89 and 3.88 (two s, 12H, 4 x OCH_3), 3.76 (ddd, $J_{4",5"} = 9.3$,

$J_{5",6"A}$ = 4.9, $J_{5",6"B}$ = 2.4, 2H, 2 x H-5"), 2.08, 2.05, and 2.04 (three s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.7, 170.3, 169.5, and 169.4 (8 x CO), 154.1 and 153.9 (2 x C-2',5'), 132.4 and 131.6 (2 x C-2,3,5,6), 124.1 and 121.6 (2 x C-1,4), 115.7 and 115.5 (2 x C-3',6'), 113.5 and 112.6 (2 x C-1',4'), 98.2 (2 x C-1"), 94.6, 89.2, 88.2, 83.4, 82.1, and 77.2 (6 x C≡C), 72.8 (2 x C-3"), 71.9 (2 x C-5"), 71.1 (2 x C-2"), 68.3 (2 x C-4"), 61.8 (2 x C-6"), 57.0 (2 x $\underline{C}H_2C\equiv$), 56.5 and 56.3 (4 x OCH_3), 20.7 and 20.6 (8 x $\underline{C}H_3CO$). Anal. Calcd for $C_{70}H_{66}O_{24}$ (1291.26): C, 65.11; H, 5.15. Found: C, 65.26; H, 5.13.

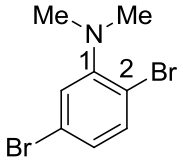
Compound 14. It was obtained following **procedure A** starting from **12** (0.23g), as a pale yellow solid (0.15g, 0.19 mmol, 94%).



14

TLC: R_f 0.05 ($CHCl_3$/MeOH 80:20). Mp ≥280 °C. ^1H NMR (dmso-*d6*): δ 7.58 (s, 4H, H-2,3,5,6), 7.18 and 7.09 (two s, 4H, 2 x H-3',6'), 5.15 (d, J_{vic} = 4.9, 2H, 2 x OH), 4.98 (d, J_{vic} = 4.9, 2H, 2 x OH), 4.94 (d, J_{vic} = 5.3, 2H, 2 x OH), 4.68 and 4.55 (AB system, J_{gem} = 15.6, 4H, 2 x $CH_2C\equiv$), 4.54 (t, $J_{OH,6}$= 5.7, 2H, 2 x 6'-OH), 4.34 (d, $J_{1",2"}$ = 7.9, 2H, 2 x H-1"), 3.82 and 3.81 (two s, 12H, 4 x OCH_3), 3.70 and 3.45 (split AB m, 4H, 2 x H_2-6"), 3.19 - 2.97 (m, 8H, 2 x H-2"-5"). ^{13}C NMR (dmso-*d6*): δ 153.7 and 153.5 (2 x C-2',5'), 131.6 (s, C-2,3,5,6), 122.5 (C-1,4), 115.8 and 115.6 (2 x C-3',6'), 112.5 and 112.2 (2 x C-1',4'), 101.1 (2 x C-1"), 94.1, 91.4, 88.1.2 and 82.0 (4 x C≡C), 77.0 and 76.7 (2 x C-3",5"), 73.3 (2 x C-2"), 70.1 (2 x C-4"), 61.2 (2 x C-6"), 56.3 and 56.1 (4 x OCH_3), 55.8 (2 x $\underline{C}H_2C\equiv$). Anal. Calcd for $C_{44}H_{46}O_{16}$ (830.83): C, 63.61; H, 5.58. Found: C, 63.63; H, 5.59.

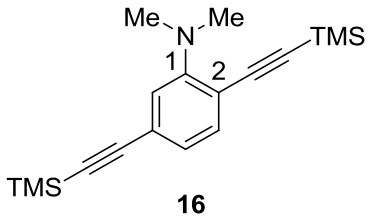
Compound 15. A DMF solution (12 mL) of 2,5-dibromoaniline (1.00 g, 3.98 mmol, 1 equiv) was added at RT under Ar atmosphere to anhydrous K_2CO_3 (5.00 g, 36.18 mmol). To the obtained suspension 1.2 mL of iodomethane (20 mmol, 5 equiv) were added, the mixture was heated at 100 °C, and maintained in these conditions under continuous stirring until completion by TLC (hexane/EtOAc 9.5:0.5). After 48 h, the reaction was quenched by adding water (7 mL) and extracted with dichloromethane (3 x 10 mL). The organic phases were dried over anhydrous Na_2SO_4, the solvent evaporated under reduced pressure to give pure **15** as a transparent oil (1.08 g, 3.87 mmol, 97%).



15

TLC: R_f 0.85 (hexane/EtOAc 95:5). ^{1}H NMR: δ 7.35 (d, 1H, $J_{3,4}$ = 8.8, H-3), 7.14 (d, 1H, $J_{4,6}$ = 2.4, H-6), 6.96 (dd, 1H, $J_{3,4}$ = 8.8, $J_{4,6}$ = 2.4, H-4), 2.76 [s, 6H, $N(CH_3)_2$]. ^{13}C NMR: δ 152.8 (C-1), 134.7 (C-3), 126.2 (C-4), 123.5 (C-6), 119.9 and 117.2 (C-4,5), 43.7 [$N(CH_3)_2$]. Anal. Calcd for $C_8H_9Br_2N$ (278.97): C, 34.44; H, 3.25; N, 5.02. Found: C, 34.50; H, 3.26; N, 5.03.

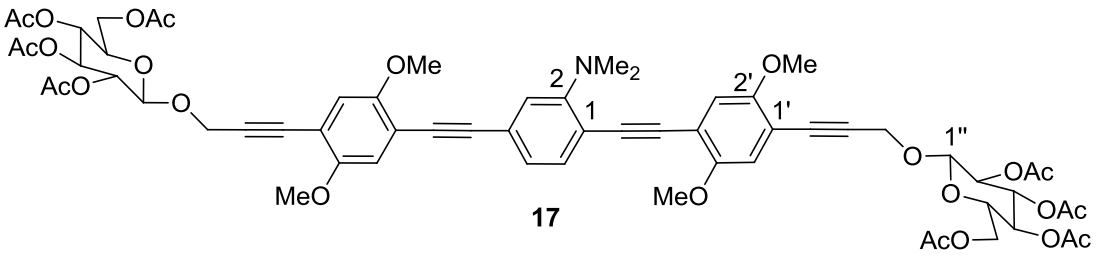
Compound 16. To a solution in dry DMF (15 mL) of **15** (1.00 g, 3.58 mmol, 1 equiv), commercial ethynyltrimethylsilane (3.05 mL, 21.60 mmol, 6 equiv) and $Pd(PPh_3)_4$ (0.41 g, 0.35 mmol, 0.1 equiv) Et_3N (15 mL) was slowly added. The mixture was heated at 70 °C and maintained under Ar atmosphere and continuous stirring until the disappearance of starting products (after 2 hours) by TLC (hexane/EtOAc 9:1). Column chromatography was performed with hexane as eluant, and compound **16** was obtained as a transparent oil (0.62 g, 1.98 mmol, 55%).



16

TLC: R_f 0.67 (hexane/EtOAc 90:10). ^{1}H NMR: δ 7.33 (d, 1H, $J_{3,4}$ = 7.9, H-3), 6.94 (m, 2H, H-4,6), 2.93 [s, 6H, $N(CH_3)_2$], 0.25 [s, 18H, 2 x $Si(CH_3)_3$]. ^{13}C NMR: δ 154.7 (C-1), 134.6 (C-3), 123.8 and 120.1 (C-4,6), 115.0 and 105.1 (C-2,5), 104.3, 104.2, 101.4, and 95.3 (2 x C≡C), 43.1 [$N(CH_3)_2$], - 0.02 and - 0.17 [2 x $Si(CH_3)_3$]. Anal. Calcd for $C_{18}H_{27}NSi_2$ (313.58): C, 68.94; H, 8.68; N, 4.47. Found: C, 68.88; H, 8.70; N, 4.48.

Compound 17. Compounds **16** (0.18 g, 0.57 mmol, 1 equiv) and **4** (0.75 g, 1.16 mmol, 2 equiv), Ag_2O (0.27 g, 1.16 mmol, 2 equiv) and $Pd(PPh_3)_4$ (0.10 g, 0.09 mmol, 0.16 equiv) were suspended in dry DMF (4 mL) and THF (2 mL). The obtained mixture was heated at 70 °C and maintained under Ar atmosphere and continuous stirring for 7h, until the disappearance of starting products by TLC (hexane/EtOAc 3:7) After filtration over Celite, the solvents were removed under reduced pressure, and the obtained reaction crude was subjected to silica gel column chromatography Column chromatography was performed with hexane/EtOAc 50:50 as eluant, and compound **17** was obtained as a brilliant yellow solid (0.42 g, 0.35 mmol, 61%).

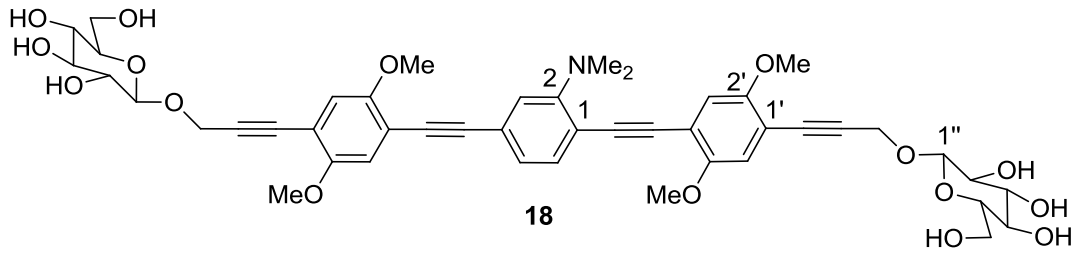


17

TLC: R_f 0.56 (hexane/EtOAc 40:60). Mp 91-93 °C.^{1}H NMR: δ 7.46 (d, $J_{5,6}$ = 8.4, 1H, H-6), 7.08 (m, 2H, H-3,5), 7.00, 6.99, 6.93 and 6.91 (four s, 4H, 2 x H-3',6'), 5.25 (t, $J_{2",3"} = J_{3",4"}$ = 9.5, 2H, 2 x H-3"), 5.10 (t, $J_{3",4"} = J_{4",5"}$ = 9.5, 2H, 2 x H-4"), 5.03 (dd, $J_{1",2"}$ = 8.0, $J_{2",3"}$ = 9.5, 2H, 2 x H-

2"), 4.90 (d, $J_{1",2"}$ = 8.0, 2H, 2 x H-1"), 4.64 (s, 4H, 2 x $CH_2C\equiv$), 4.27 and 4.16 (split AB system, $J_{5",6"A}$ = 4.9, $J_{5",6"B}$ = 2.4, $J_{6"A,6"B}$ = 12.7, 4H, 2 x H_2-6"), 3.87, 3.86, and 3.85 (three s, 12H, 4 x OCH_3), 3.76 (ddd, $J_{4",5"}$ = 9.5, $J_{5",6"A}$ = 4.9, $J_{5",6"B}$ = 2.4, 2H, 2 x H-5"), 3.02 [br s, 6H, $N(CH_3)_2$], 2.06, 2.03, 2.02, and 2.01 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.7, 170.3, 169.5, and 169.4 (8 x CO), 154.5, 154.1, 153.9, and 153.8 (C-2, 2 x C-2',5'), 134.3 (C-6), 128.2 (C-4), 123.7 and 120.0 (C-3,5), 115.7, 115.6, 115.5, and 115.1 (2 x C-3',6'), 115.0, 114.4, 113.8, 112.3, and 111.9 (C-1, 2 x C-1',4'), 98.3 (2 x C-1"), 95.5, 94.6, 92.6, 89.1, 88.9, 86.6, 83.5, and 83.4 (4 x C≡C), 72.8 (2 x C-3"), 71.9 (2 x C-5"), 71.1 (2 x C-2"), 68.3 (2 x C-4"), 61.8 (2 x C-6"), 57.1 (2 x $\underline{C}H_2C\equiv$), 56.5, 56.4 and 56.3 (4 x OCH_3), 43.5 [$N(CH_3)_2$], 20.7 and 20.6 (8 x $\underline{C}H_3CO$). Anal. Calcd for $C_{62}H_{67}NO_{24}$ (1210.19): C, 61.53; H, 5.58; N, 1.16. Found: C, 61.66; H, 5.57; N, 1.16.

Compound 18. It was obtained following **procedure A** starting from **17** (0.24g), as a pale yellow solid (0.17g, 0.19 mmol, 95%).

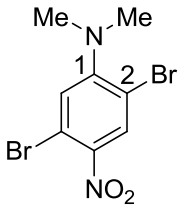


18

TLC: R_f 0.05 ($CHCl_3$/MeOH 80:20). Mp 202-203 °C. ^{1}H NMR (dmso-*d6*): δ 7.44 (d, $J_{5,6}$ = 8.3, 1H, H-6), 7.16, 7.10, 7.07 and 7.06 (four s, 4H, 2 x H-3',6'), 7.01 (m, 2H, H-3,5), 5.14 (d, J_{vic} = 4.9, 2H, 2 x OH), 4.97 (d, J_{vic} = 4.8, 2H, 2 x OH), 4.92 (d, J_{vic} = 5.4, 2H, 2 x OH), 4.67 and 4.54 (AB system, J_{gem} = 16.1, 4H, 2 x $CH_2C\equiv$), 4.56 (t, $J_{OH,6}$ = 5.9, 2H, 2 x 6'-OH), 4.33 (d, $J_{1",2"}$ = 7.8, 2H, 2 x H-1"), 3.81 and 3.80 (two s, 12H, 4 x OCH_3), 3.66 and 3.44 (split AB m, 4H, 2 x H_2-6"), 3.18 - 2.97 (m, 8H, 2 x H-2"-5"), 2.97 [s, 6H, $N(CH_3)_2$]. ^{13}C NMR (dmso-*d6*): δ 154.1, 153.8, 153.7, and 153.5 (C-2, 2 x C-2',5'), 134.5 (C-6), 123.3 (C-4), 122.7 and 119.2 (C-3,5), 115.8, 115.6, and 115.0 (2 x C-3',6'), 113.7, 113.2, 112.5, 112.4, and 112.0 (C-1, 2 x C-1',4'),

101.1 (2 x C-1"), 94.8, 94.3, 92.8, 91.3, 91.2, 87.3, 82.1, and 82.0 (4 x C≡C), 77.1 and 76.7 (2 x C-3",5"), 73.3 (2 x C-2"), 70.1 (2 x C-4"), 61.2 (2 x C-6"), 56.3 and 56.2 (4 x OCH_3), 55.9 (2 x $\underline{C}H_2C\equiv$), 42.7 [$N(CH_3)_2$]. Anal. Calcd for $C_{46}H_{51}NO_{16}$ (873.89): C, 63.22; H, 5.88; N, 1.60. Found: C, 63.25; H, 5.87; N, 1.60.

Compound 22.[3] A DMF solution (5 mL) of **21** (0.40 g, 1.35 mmol, 1 equiv) was added at RT under Ar atmosphere to anhydrous K_2CO_3 (2.38 g, 17.22 mmol). To the obtained suspension 0.41 mL of iodomethane (6.75 mmol, 5 equiv) were added, the mixture was heated at 100 °C, and maintained in these conditions under continuous stirring until completion by TLC (hexane/EtOAc 9:1). After 70 h, the reaction was quenched by adding water (5 mL) and extracted with dichloromethane (3 x 10 mL). The organic phases were dried over anhydrous Na_2SO_4, the solvent evaporated under reduced pressure. The reaction crude was subjected to silica gel column chromatography (eluant hexane/EtOAc 90:10) and **22** has been isolated as a yellow solid (0.26 g, 0.80 mmol, 59%). TLC: R_f 0.80 (hexane/EtOAc 80:20).



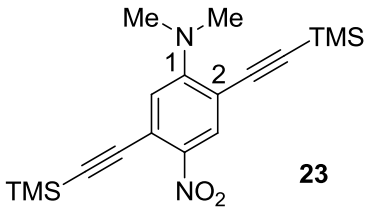
22

Mp 81-83 °C, Rf=0.85.

Compound 23. In a flask containing **22** (0.25 g, 0.77 mmol, 1 equiv) were added commercial ethynyltrimethylsilane (0.65 mL, 4.62 mmol, 6 equiv), $Pd(PPh_3)_4$ (0.09 g, 0.08 mmol, 0.1 equiv). The compounds were dissolved in dry DMF (6 mL) and to the solution was slowly added dry Et_3N (6 mL). The mixture was heated at 70 °C and maintained under Ar atmosphere and

[3] Moroni et al.; *Macromolecules*, **1997**, 30, 1964-1972.

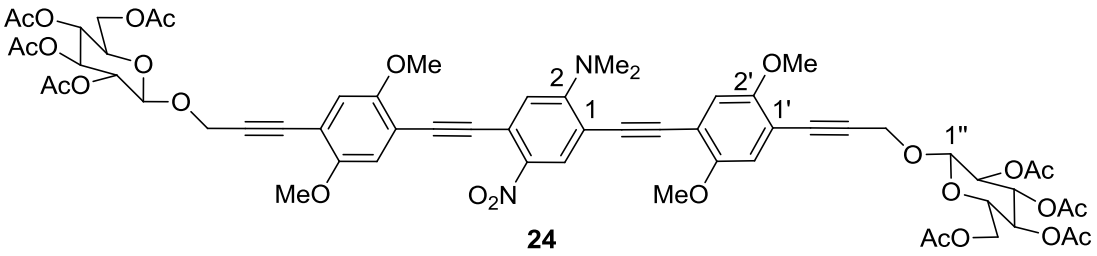
continuous stirring 1h until the disappearance of starting products. Column chromatography was performed with hexane as eluant, and compound **23** was obtained as a yellow oil (0.19 g, 0.53 mmol, 69%).



23

TLC: R_f 0.48 (hexane/EtOAc 80:20).^{1}H NMR: δ 8.19 (s, 1H, H-3), 6.85 (s, 1H, H-6), 3.16 [s, 6H, $N(CH_3)_2$], 0.28 and 0.24 [two s, 18H, 2 x $Si(CH_3)_3$]. ^{13}C NMR: δ 156.1 (C-1), 139.6 (C-4), 133.1 (C-3), 120.7 (C-6), 119.7 and 110.7 (C-2,5), 103.7, 102.6, 102.5, and 100.8 (2 x C≡C), 42.5 [$N(CH_3)_2$], - 0.27 and - 0.42 [2 x $Si(CH_3)_3$]. Anal. Calcd for $C_{18}H_{26}N_2O_2Si_2$ (358.58): C, 60.29; H, 7.31; N, 7.81. Found: C, 60.40; H, 7.29; N, 7.78.

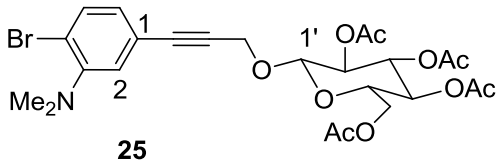
Compound 24. **23** (0.22 g, 0.61 mmol, 1 equiv), **4** (0.75 g, 1.16 mmol, 2 equiv.), $Pd(PPh_3)_4$ (0.10 g, 0.09 mmol, 0.15 equiv), and Ag_2O (0.27 g, 1.16 mmol, 2 equiv) were suspended in dry DMF (4 mL) and THF (2 mL). The obtained mixture was heated at 70 °C for 30h and maintained under Ar atmosphere and continuous stirring until the disappearance of starting products. After filtration over Celite, solvents were removed under reduced pressure, and the reaction crude was subjected to silica gel column chromatography, performed with hexane/EtOAc 50:50 as eluant: compound **24** was obtained as a pale green oil (0.31 g, 0.25 mmol, 41%). TLC: R_f 0.36 (hexane/EtOAc 40:60).



24

^{1}H NMR: δ 8.38 (s, 1H, H-6), 7.10 (s, 1H, H-3), 6.98, 6.96, and 6.93 (three s, 4H, 2 x H-3',6'), 5.27 (t, $J_{2",3"} = J_{3",4"}$ = 9.4, 2H, 2 x H-3"), 5.12 (t, $J_{3",4"} = J_{4",5"}$ = 9.4, 2H, 2 x H-4"), 5.05 (dd, $J_{1",2"}$ = 8.2, $J_{2",3"}$ = 9.4, 2H, 2 x H-2"), 4.90 (d, $J_{1",2"}$ = 8.2, 2H, 2 x H-1"), 4.65 (s, 4H, 2 x $CH_2C{\equiv}$), 4.27 and 4.16 (split AB system, $J_{5",6"A}$ = 4.1, $J_{5",6"B}$ = 2.3, $J_{6"A,6"B}$ = 12.3, 4H, 2 x H_2-6"), 3.91, 3.89, and 3.86 (three s, 12H, 4 x OCH_3), 3.78 (ddd, $J_{4",5"}$ = 9.4, $J_{5",6"A}$ = 4.1, $J_{5",6"B}$ = 2.3, 2H, 2 x H-5"), 3.28 [s, 6H, $N(CH_3)_2$], 2.08, 2.05, 2.03, and 2.01 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.2, 169.4, and 169.3 (8 x CO), 155.6, 154.3, 154.1, and 154.0 (C-2 and 2 x C-2',5'), 139.0 (C-5), 133.0 (C-6), 120.1, 119.9, 115.8, 115.4, and 114.9 (C-3 and 2 x C-3',6'), 113.4, 113.2, 112.5, and 110.5 (C-1 and 2 x C-1',4'), 98.2 (2 x C-1"), 93.3, 93.1, 93.0, 92.1, 89.5, 89.3, and 83.3 (4 x C≡C), 72.8 (2 x C-3"), 71.9 (2 x C-5"), 71.1 (2 x C-2"), 68.3 (2 x C-4"), 61.8 (2 x C-6"), 57.0 (2 x $\underline{C}H_2C{\equiv}$), 56.5, 56.4, and 56.2 (4 x OCH_3), 42.8 [$N(CH_3)_2$], 20.7 and 20.6 (8 x $\underline{C}H_3CO$). Anal. Calcd for $C_{62}H_{66}N_2O_{26}$ (1255.19): C, 59.33; H, 5.30; N, 2.23. Found: C, 59.26; H, 5.29; N, 2.23.

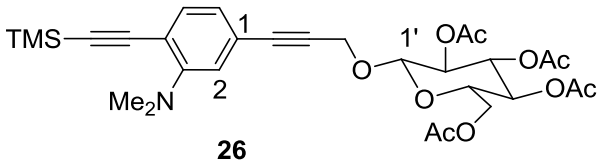
Compound 25. To a flask were added $Pd(PPh_3)_4$ (0.45g, 0.39mmol, 0.15 equiv), **1** (1.00g, 2.59mmol, 1equiv) and **16** (1.45g, 5.18mmol, 2 equiv); the flask was capped with a rubber septum and evacuated. After backfilling with N_2, this process was repeated three times. To the flask were added dry DMF (20mL) and Et_3N (20mL) at room temperature. The reaction mixture was heated at 65°C, and maintained under continuous stirring for 4 h, until the disappearance of compound **1** by TLC (hexane/EtOAc 7:3) (Hexane/EtOAc 70:30). Solvents were removed under reduced pressure and the solid residue was dissolved in CH_2Cl_2 ad filtered on celite. The volatiles were removed *in vacuo* and the reaction crude was purified by flash chromatography on silica gel, using Hexane/EtOAc (90:10) as eluant, giving compound **25** as a pale yellow oil (0.91g, 1.55mmol, 60%).



25

TLC: *Rf*0.60. ^{1}H NMR: δ 7.48 (d, $J_{5,6}$ = 8.1, 1H, H-5), 7.11 (d, $J_{2,6}$ = 1.0, 1H, H-2), 6.93 (dd, $J_{5,6}$ = 8.1, $J_{2,6}$ = 1.0, 1H, H-6), 5.25 (t, $J_{2',3'} = J_{3',4'}$ = 9.3, 1H, H-3'), 5.10 (t, $J_{3',4'} = J_{4',5'}$ = 9.3, 1H, H-4'), 5.02 (*b*dd, $J_{1',2'}$= 8.3 $J_{2',3'}$ = 9.3, 1H, H-2'), 4.80 (d, $J_{1',2'}$ = 8.3, 1H, H-1'), 4.56 (s, 2H, $CH_2C\equiv$), 4.27 and 4.14 (split AB system, $J_{5',6A'}$= 4.2, $J_{5',6B'}$ = 1.8, $J_{6A',6B'}$ = 12.6, 2H, H_2-6'), 3.79 (m, 1H, H-5'), 2.78 (s, 6H, [$N(CH_3)_2$]), 2.06, 2.03, 2.01 and 1.99 (four s, 12H, 4 x CH_3CO). ^{13}C NMR: δ 170.6, 170.2 and 169.4 (4 x CO), 151.9 (C-3), 134.0 (C-5), 126.8 and 123.6 (C-2 and C-6), 121.9 and 119.9 (C-1 and C-4), 98.4 (C-1'), 86.3 and 83.9 (C≡C), 72.7 (C-3'), 71.8 (C-5'), 71.1 (C-2'), 68.2 (C-4'), 61.8 (C-6'), 56.8 ($\underline{C}H_2C\equiv$), 44.0 ([$N(CH_3)_2$]), 20.6 and 20.5 (4 x $\underline{C}H_3CO$). Calc. for $C_{25}H_{30}BrNO_{10}$ 583.11; found positive ESI-MS: [M-H+] = 584.1114**.**

Compound 26. To a flask were added $Pd(PPh_3)_4$ (0.27g, 0.23mmol, 0.15equiv), **25** (0.91g, 1.55mmol, 1equiv) and ethynyltrimethylsilane (1.31mL, 9.3mmol, 6 equiv); the flask was capped with a rubber septum and evacuated. After backfilling with N_2, this process was repeated three times. To the flask were added dry DMF (11 mL) and Et_3N (11 mL) at room temperature. The mixture was heated at 65°C, and maintained under continuous stirring for 24h, until the disappearance of compound **25** by TLC (Hexane/EtOAc 7:3). Solvents were removed *in vacuo* and the solid residue was dissolved in CH_2Cl_2 ad filtered on celite. The volatiles were removed under reduced pression and the crude purified by flash chromatography on silica gel using Hexane/EtOAc (90:10) as eluant to give compound **26** as a yellow oil (0.77g, 1.27mmol, 82%).

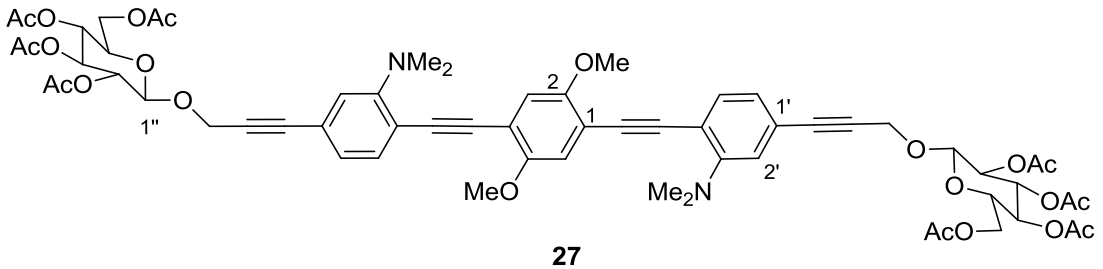


26

TLC: *Rf*0.55. ^{1}H NMR: δ 7.31 (d, $J_{5,6}$ = 7.8, 1H, H-5), 6.86 (d, $J_{2,6}$ = 1.2, 1H, H-2), 6.87 (dd, $J_{5,6}$

= 7.8, $J_{2,6}$ = 1.2, 1H, H-6), 5.24 (t, $J_{2',3'} = J_{3',4'} =$ 9.3, 1H, H-3'), 5.09 (t, $J_{3',4'} = J_{4',5'}$ = 9.3, 1H, H-4'), 5.01 (*b*dd, $J_{1',2'}$= 8.1 $J_{2',3'}$ = 9.3, 1H, H-2'), 4.82 (d, $J_{1',2'}$ = 8.1, 1H, H-1'), 4.57 (s, 2H, $CH_2C\equiv$), 4.27 and 4.15 (split AB system, $J_{5',6A'}$ = 4.5, $J_{5',6B'}$ = 2.1, $J_{6A',6B'}$ = 12.3, 2H, H_2-6'), 3.74 (m, 1H, H-5'), 2.94 (s, 6H, [$N(CH_3)_2$]), 2.06, 2.03, 2.01 and 1.99 (four s, 12H, 4 x CH_3CO), 0.24 (s, 9H, [$Si(CH_3)_3$]). ^{13}C NMR: δ 170.6, 170.2, 169.4 and 169.3 (4 x CO), 154.8 (C-3), 134.8 (C-5), 123.2 and 119.8 (C-2 and C-6), 122.8 and 116.2 (C-1 and C-4), 104.0, 101.7, 87.2 and 84.4 (C≡C), 98.4 (C-1'), 72.8 (C-3'), 71.9 (C-5'), 71.1 (C-2'),68.3 (C-4'), 61.8 (C-6'), 56.9 ($\underline{C}H_2C\equiv$), 43.1 [$N(CH_3)_2$], 21.0, 20.7 and 20.5 (4 x $\underline{C}H_3CO$), 0.2 ([$Si(CH_3)_3$]). Calc. for $C_{30}H_{39}NO_{10}Si$ 601.23; found positive ESI-MS: [M-H+] = 602.2422.

Compound 27. To a flask were added $Pd(PPh_3)_4$ (0.11g, 0.09mmol, 0.15 equiv), Ag_2O (0.30 g, 1.28 mmol, 2equiv), **26** (0.77g, 1.28mmol, 2equiv) and 1,4-iodo-2,5-dimethoxybenzene **2** (0.25g, 0.64mmol, 1 equiv); the flask was capped with a rubber septum and evacuated. After backfilling with N_2, this process was repeated three times. To the flask were added dry DMF (10mL) and dry THF (5mL). The reaction mixture was heated at 70°C for 7 h, until the disappearance of compound **25** by TLC (Hexane/EtOAc 1:1). Solvents were removed *in vacuo* and the solid residue was dissolved in CH_2Cl_2 ad filtered on celite. The volatiles were removed under reduced pression and the crude purified by column chromatography on silica gel using Hexane/EtOAc (60:40) as eluant to give compound **27** as a brilliant yellow oil (0.43g, 0.36mmol, 57%).

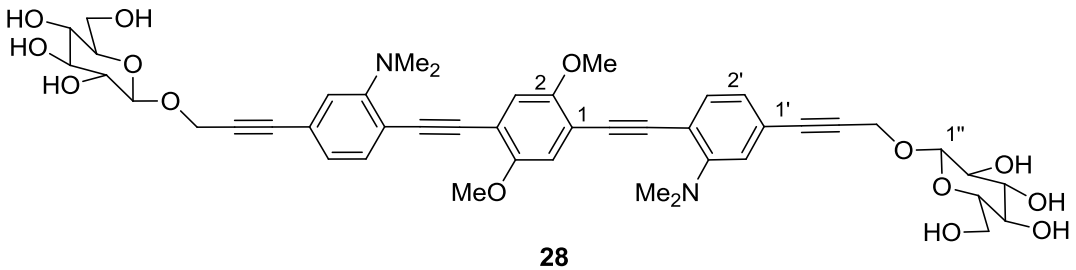


27

TLC: *Rf*0.35. ^{1}H NMR: δ 7.45 (d, $J_{5',6'}$ = 7.8, 2H, 2 x H-5'), 6.99 (s, 2H, H-3 and H-6), 6.97 (d,

$J_{2',6'}$ = 1.2, 2H, 2xH-2'), 6.96 (dd, $J_{5',6'}$ = 7.8, $J_{2',6'}$ = 1.2, 2H, 2xH-6'), 5.28 (t, $J_{2'',3''} = J_{3'',4''}$ = 9.3, 2H, 2xH-3''), 5.12 (t, $J_{3'',4''} = J_{4'',5''}$ = 9.3, 2H, 2xH-4''), 5.04 (*b*dd, $J_{1'',2''}$= 8.2 $J_{2'',3''}$ = 9.3, 2H, 2xH-2''), 4.85 (d, $J_{1'',2''}$ = 8.2, 2H, 2xH-1''), 4.60 (s, 4H, 2x$CH_2C{\equiv}$), 4.29 and 4.17 (split AB system, $J_{5'',6A''}$ = 4.4, $J_{5'',6B''}$ = 2.5, $J_{6A'',6B''}$ = 12.2, 4H, 2xH_2-6''), 3.89 (s, 6H, 2xOCH_3), 3.77 (m, 2H, 2xH-5''), 3.03 (s, 12H, 2x[$N(CH_3)_2$]), 2.08, 2.05, 2.03 and 2.02 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.6, 170.2, 169.4 and 169.3 (8 x CO), 154.3 and 153.9 (C-2, C-5, C-3'), 134.3 (C-5'), 123.5 (C-6'), 122.6 (C-4'), 119.9 (C-2'), 115.5, 114.9 and 114.8 (C-1, C-3, C-4 and C-6), 113.6 (C-1'), 98.4 (C-1''), 98.1, 93.0, 87.2 and 84.4 (C≡C), 72.7 (C-3''), 71.8 (C-5''), 71.1 (C-2''),68.3 (C-4''), 61.7 (C-6''), 56.9 ($\underline{C}H_2C{\equiv}$), 56.3 ($OCH_3$), 43.3 [$N(CH_3)_2$], 20.7 and 20.5 (8 x $\underline{C}H_3CO$). Calc. for $C_{62}H_{68}N_2O_{22}$ 1192.43: found positive MALDI [M+] = 1192.4; Purity by qNMR: 96.3%.%.

Compound 28. It was obtained following **procedure A** starting from **27** (0.43g, 0.36mmol) with the final obtaining of compound **28** as a brilliant yellow solid (0.30g, 0.35mmol, 97%).

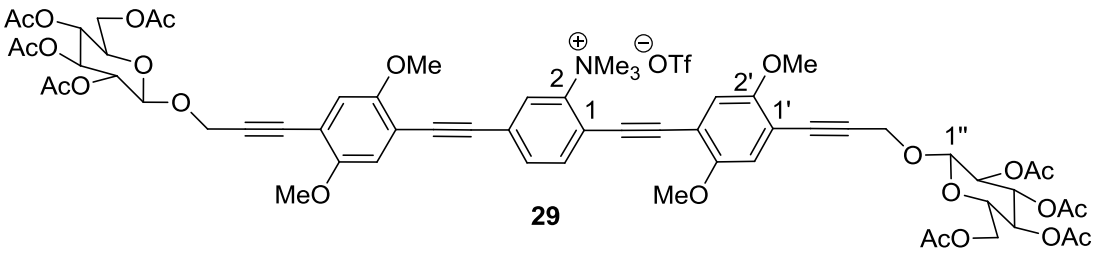


28

TLC: *Rf*0.05 ($CHCl_3$/MeOH 80:20). Mp 123-125°C. ^{1}H NMR (dmso-*d6*): δ 7.40 (d, $J_{5',6'}$ = 8.7, 2H, 2xH-5'), 7.12 (s, 2H, H-3 and H-6), 6.95 (m, 4H, 2xH-2' and 2xH-6'), 5.14 (d, $J_{2'',OH}$ = 4.4, 2H, 2xOH-2''), 4.97 and 4.92 (two d, $J_{3'',OH} = J_{4'',OH}$ =4.4, 4H, 2xOH-3'' and 2xOH-4''), 4.64 and 4.51 (AB system and m, J_{gem}=15.7, 6H, 2 x $CH_2C{\equiv}$ and 2 x OH-6''), 4.31 (d, 2H, $J_{1'',2''}$ = 7.8, 2xH-1''), 3.83 (s, 6H, 2xOCH_3), 3.70 and 3.46 (split AB m, 4H, 2 x H_2-6''), 3.16-2.98 (m, 8H, 2xH-2'', 2xH-3'', 2xH- 4'', 2xH-5''), 2.96 (s, 12H, 2x[$N(CH_3)_2$]). ^{13}C NMR (dmso-*d6*): δ 153.9

and 153.5 (C-2, C-5 and C-3'), 134.4 (C-5'), 131.5 (C-1'), 122.8, 122.6, 119.5 and 119.4 (C-2'and C-6'), 114.9 and 114.8 (C-3 and C-6), 113.7 and 112.9 (C-1, C-4 and C-4'), 101.2 (C-1''), 94.2, 92.8, 87.1 and 85.6 (C≡C), 77.0 and 76.5 (C-3'' and C-5''), 73.3 (C-2''), 70.0 (C-4''), 61.3 (C-6''), 56.2 ($\underline{C}H_2C\equiv$), 55.7 (OCH_3), 42.6 [$N(CH_3)_2$]. Calc. for $C_{46}H_{52}N_2O_{14}$ 856.34; found positive ESI-MS: [M-H+] = 857.3515; Purity by qNMR: 96.3%.

Compound 29. Compound **17** (0.50g, 0.41mmol, 1eq) was dissolved in dry dichloromethane. To this solution a equimolar amount of MeOTf (0.07g, 0.41mmol, 1eq) was added under Ar atmosphere. The mixture was heated at reflux temperature (40°) and maintained under continuous stirring until the disappearance of the starting product **17** by TLC (hexane/EtOAc 3:7) (2h). Solvents were removed under reduced pressure to give **29** as an orange solid (0.49g, 0.40mmol, 97%).

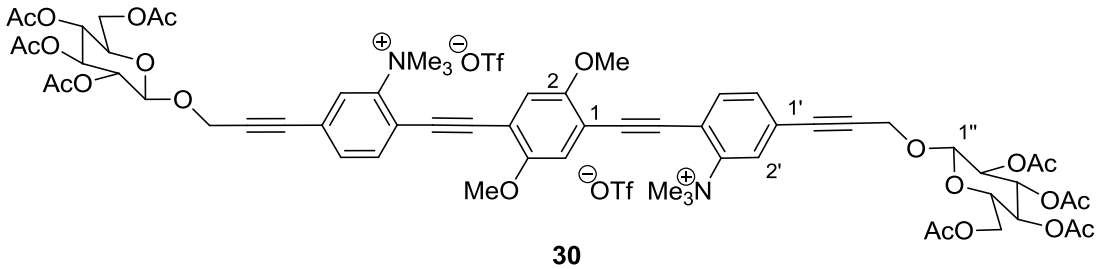


29

TLC: *Rf*: (0.10). Mp 162-164 °C. ^{1}H NMR: δ 7.89 (d, $J_{3,5}$ = 1.0, 1H, H-3), 7.78 (d, $J_{5,6}$ = 7.8, 1H, H-6), 7.70 (dd, $J_{3,5}$ = 1.0, $J_{5,6}$ = 7.8, 1H, H-5), 7.07, 6.98, and 6.96 (three s, 4H, 2 x H-3',6'), 5.27 (t, $J_{2'',3''}$ = $J_{3'',4''}$ = 9.3, 2H, 2 x H-3''), 5.12 (t, $J_{3'',4''}$ = $J_{4'',5''}$ = 9.3, 2H, 2 x H-4''), 5.04 (*broad* dd, $J_{1'',2''}$ = 7.9, $J_{2'',3''}$ = 9.3, 2H, 2 x H-2''), 4.90 (d, $J_{1'',2''}$ = 7.8, 2H, 2 x H-1''), 4.66 and 4.65 (two s, 4H, 2 x $CH_2C\equiv$), 4.28 and 4.17 (split AB m, 4H, 2 x H_2-6''), 4.09 [(s, 9H, $N(CH_3)_3$], 3.95, 3.90, 3.89, and 3.88 (four s, 12H, 4 x OCH_3), 3.77 (m, 2H, 2 x H-5''), 2.08, 2.05, 2.03, and 2.01 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.7, 170.3, 169.5, and 169.4 (8 x CO), 155.2 and 154.1 (2 x C-2',5'), 145.1 (C-2), 137.1 (C-6), 133.1 (C-5), 125.9 (C-4), 122.9 (C-3), 115.7, 115.5, 114.8,

114.5, 113.6, 112.2, and 110.8 (C-1, 2 x C-1',3',4',6'), 100.2, 92.2, 91.3, 90.5, 89.8, 88.7, 83.2, and 82.9 (4 x C≡C), 98.3 (2 x C-1"), 72.8 (2 x C-3"), 71.9 (2 x C-5"), 71.1 (2 x C-2"), 68.3 (2 x C-4"), 61.8 (2 x C-6"), 57.1 and 57.0 (2 x $\underline{C}H_2C\equiv$), 56.5, 56.4, and 56.3 (4 x OCH_3), 56.0 [$N(CH_3)_3$], 20.7 and 20.6 (8 x $\underline{C}H_3CO$). Anal. Calcd for $C_{64}H_{70}F_3NO_{27}S$ (1374.29): C, 55.93; H, 5.13; N, 1.02. Found: C, 56.03; H, 5.12; N, 1.02.

Compound 30. To compound **27** (0.40g, 0.33 mmol, 1eq) dissolved in dry CH_2Cl_2 (2mL).was added, under Ar atmosphere, methyl trifluoromethanesulfonate (0.11g, 0.66mmol, 2eq). The mixture was heated at reflux temperature (40°) and maintained under continuous stirring until the disappearance of the starting product by TLC (hexane/EtOAc 3:7) (6h). Solvents were removed under reduced pressure to give **30** a yellow-orange solid (0.38g, 0.31mmol, 94%).

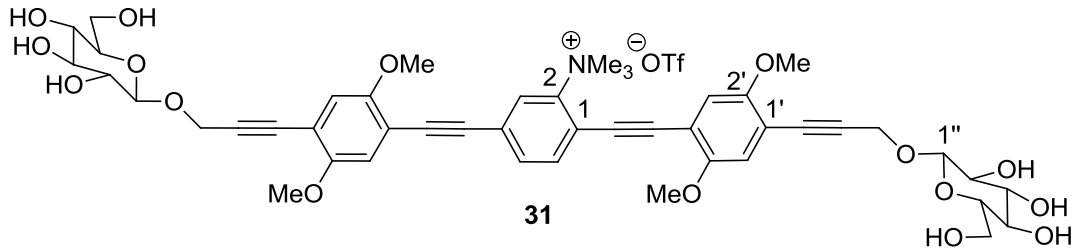


30

Mp 210°-213° TLC *Rf* (0.10) ^{1}H NMR: δ 7.90 (s, 2H, 2xH-2'), 7.70 (dd, $J_{5',6'}$ = 7.8, $J_{2',6'}$ = 1.2, 2H, 2xH-6'), 7.57 (m, 2H, 2 x H-5'), 7.07 (s, 2H, H-3 and H-6), 5.26 (t, $J_{2'',3''} = J_{3'',4''}$ = 9.3, 2H, 2xH-3''), 5.12 (t, $J_{3'',4''} = J_{4'',5''}$ = 9.3, 2H, 2xH-4''), 5.00 (*b*dd, $J_{1'',2''}$= 8.2 $J_{2'',3''}$ = 9.3, 2H, 2xH-2''), 4.84 (d, $J_{1'',2''}$ = 8.2, 2H, 2xH-1''), 4.62 (s, 4H, 2x$CH_2C\equiv$), 4.27 and 4.17 (split AB system, $J_{5'',6A''}$ = 4.4, $J_{5'',6B''}$ = 2.5, $J_{6A'',6B''}$ = 12.2, 4H, 2xH_2-6''), 4.06 (s, 18H, 2x[$N(CH_3)_3$]), 3.97 (s, 6H, 2xOCH_3), 3.83 (m, 2H, 2xH-5''), 2.06, 2.04, 2.01 and 2.00 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.7, 170.2, 169.4 (8 x CO), 155.1 and 145.1 (C-2, C-5, C-3'), 137.3 (C-5'), 133.3 (C-6'), 133.1 (C-4'), 128.4 (C-2'), 119.4, 116.1, 114.4 and 112.7 (C-1, C-3, C-4 and C-6), 113.6 (C-1'), 99.7 (C-1''), 98.8, 90.9, 89.4 and 84.3 (C≡C), 72.7 (C-3''), 71.8 (C-5''), 71.3 (C-2''),68.2

(C-4’’), 61.8 (C-6’’), 56.7 ($\underline{C}H_2C\equiv$), 56.4 (OCH_3), 56.0 [$N(CH_3)_3$], 20.7 and 20.5 (8 x $\underline{C}H_3CO$). Calc. for $C_{64}H_{74}N_2O_{22}^{2+}$

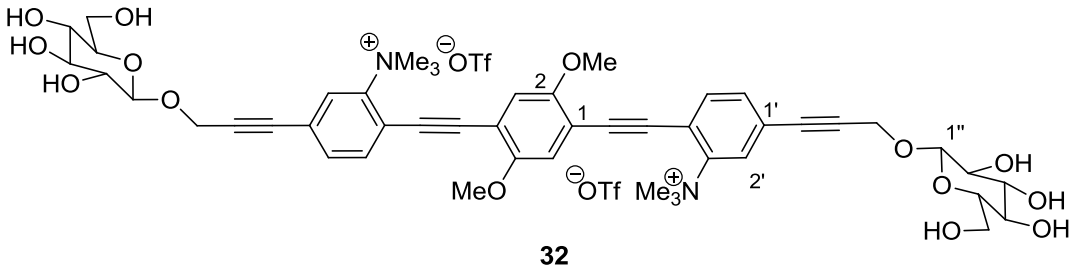
Compound 31 Following procedure A, starting from **29** (0.49g, 0.40mmol) was obtained compound **31** as a brilliant yellow solid (0.34g, 0.39mmol, 97%).



31

TLC (hexane/EtOAc 3:7), *Rf* (0.10). Mp 162-164 °C. ^{1}H NMR (dmso-*d6*): δ 8.04 (*broad* s, 1H, H-3), 7.92 (*broad* d, $J_{5,6}$ = 7.9, 1H, H-6), 7.82 (*broad* d, $J_{5,6}$ = 7.9, 1H, H-5), 7.31, 7.19, 7.15, and 7.15 (four s, 4H, 2 x H-3’,6’), 5.14 (m, 2H, 2 x OH), 4.98 (*broad* d, 2H, 2 x OH), 4.95 (*broad* d, 2H, 2 x OH), 4.61-4.53 (m, 6H, 2 x $CH_2C\equiv$ and 2 x 6’-OH), 3.92 [(s, 9H, $N(CH_3)_3$], 3.90, 3.84, and 3.82 (three s, 12H, 4 x OCH_3), 3.70 and 3.46 (split AB m, 4H, 2 x H_2-6"), 3.18 - 2.98 (m, 8H, 2 x H-2"-5"). ^{13}C NMR: 154.5, 153.8, 153.7 and 153.6 (2 x C-2’,5’), 145.1 (C-2), 136.9 (C-6), 132.9 (C-5), 124.6 (C-3), 124.1 (C-4), 116.0, 115.8, 115.6, and 115.4 (2 x C-3’,6’), 115.0, 113.9, 113.3, 111.4, and 110.8 (C-1, 2 x C-1’,3’,4’,6’), 101.1 and 101.0 (2 x C-1"), 98.8, 92.8, 92.2, 91.8, 90.3, and 81.9 (4 x C≡C), 77.1 and 76.7 (2 x C-3",-5"), 73.3 (2 x C-2"), 70.1 (2 x C-4"), 61.2 (2 x C-6"), 56.4, 56.3, and 56.2 (4 x OCH_3), 55.8 (2 x $\underline{C}H_2C\equiv$), 55.3 [$N(CH_3)_3$]. Anal. Calcd for $C_{48}H_{54}F_3NO_{19}S$ (1038.00): C, 55.54; H, 5.24; N, 1.35. Found: C, 55.55; H, 5.23; N, 1.35.

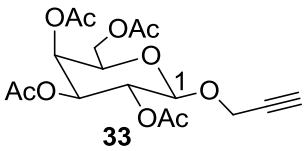
Compound 32 To a flask was added **30** (0.33g, 0.26mmol, 1eq.) and following the procedure A compound **32** afforded as a brilliant pale orange solid (0.22g, 0.24mmol, 92%).



32

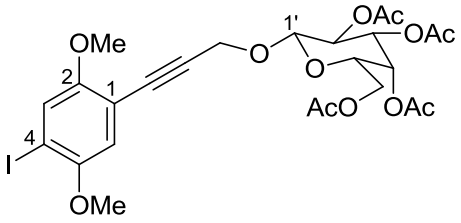
TLC(hexane/EtOAc 3:7), *Rf* (0.10) Mp 165-169 °C. ^{1}H NMR (dmso-*d6*): δ 8.01 (*broad* s, 1H, H-2'), 7.90 (*broad* d, $J_{5,6}$ = 7.9, 1H, H-6'), 7.82 (*broad* d, $J_{5,6}$ = 7.9, 1H, H-5'), 7.4 (s, 4H, 2 x H-3',6'), 5.14 (m, 2H, 2 x OH), 5.01 (*broad* d, 2H, 2 x OH), 4.96 (*broad* d, 2H, 2 x OH), 4.72-4.55 (m, 6H, 2 x $CH_2C\equiv$ and 2 x 6'-OH), 4.33 (d, 2H, 2 x H-1") 3.93 [(s, 18H, $N(CH_3)_3$], 3.90 (s, 6H, 2 x OCH_3), 3.70 and 3.46 (split AB m, 4H, 2 x H_2-6"), 3.18 - 2.98 (m, 8H, 2 x H-2"-5"). ^{13}C NMR: 155.7 and 154.8 (2 x C-2',5'), 145.2 (C-2), 137.0 (C-6), 133.2 (C-5), 125.2 (C-3), 124.0 (C-4), 116.3, 115.6, 115.3, and 115.1 (2 x C-3',6'), 114.0, 112.5, and 110.8 (C-1, 2 x C-1',3',4',6'), 101.6 and 101.5 (2 x C-1"), 98.9, 98.6, 91.0, 90.7 (4 x C≡C), 77.3, 77.0 and 76.9 (2 x C-3",-5"), 73.9 and 74.4 (2 x C-2"), 70.2 (2 x C-4"), 68.9 (2 x C-6"), 61.3 (4 x OCH_3), 58.6 (2 x $\underline{C}H_2C\equiv$), 55.6 [$N(CH_3)_3$]. Anal. Calcd for $C_{48}H_{58}N_2O_{14}^{2+}$(886.98)

33. To a solution of 1,2,3,4,6-penta-*O*-acetil-β-D-galactopyranose (5.0g, 12.55 mmol, 1eq.) in 36 mL of dry CH_2Cl_2, at 0°C and under Argon, were added 1.48 mL of propargylic alcool (25.10 mmol, d = 0.958 g/mL, 2eq.), 4.59 mL di trimethylsilyltriflate (25.10 mmol, d=1.228 g/mL, 2 eq.) and molecular sieves 4Å. The mixture was heated to RT and maintained under continuous stirring for 1h. The reaction was quenched by adding $NaHCO_3$ (40 mL) and the organic phase washed with water (2 x 30 mL). The organic phases were dried over anhydrous Na_2SO_4, the solvent evaporated under reduced pressure. Column chromatography was performed with hexane/EtOAc 70:30 as eluant, and compound **33** was obtained as a white solid (3.4 g, 70%). TLC(hexane/EtOAc 8:2): *Rf* = 0.50.



33

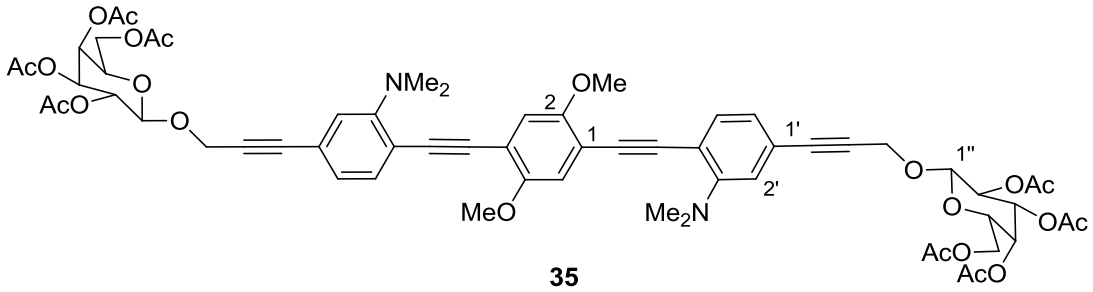
Compound 34. 2-propyn-1-yl β-D-galactopyranoside 2,3,4,6-tetraacetate **33** (1.42 g, 3.67 mmol, 1 equiv) and 1,4-diiodo-2,5-dimethoxybenzene **2** (3.00 g, 7.69 mmol, 2.1 equiv), were dissolved in dry DMF (30 mL). To th solution, at rt, was added $Pd(PPh_3)_4$ (0.50 g, 0.43 mmol, 0.12 equiv). Then to the mixture was slowly added Et_3N (30 mL). The mixture under Ar atmosphere was heated at 60 °C and maintained under continuous stirring for 3h until the disappearance of **33** by (hexane/EtOAc 7:3). Column chromatography was performed with hexane/EtOAc 70:30 as eluant, and compound **34** was obtained as a white solid (1.90 g, 2.93 mmol, 80%).



34

TLC: R_f 0.64. Mp 67-69 °C. ^{1}H NMRδ 7.29 (s, 1H, H-3), 6.85 (s, 1H, H-6), 5.40 (d, $J_{3',4'}$ = 2.0, 1H, H-4'), 5.24 (dd, $J_{2',3'} = J_{1',2'}$ = 8.0, $J_{2',4'}$ = 2.0, 1H, H-2'), 5.07 (dd, $J_{3',4'} = J_{3',2'}$ = 7.0, $J_{1',3'}$ = 3.0, 1H, H-3'), 4.84 (d, $J_{1',2'}$ = 8.0, 1H, H-1'), 4.63 (s, 2H, $CH_2C\equiv$), 4.19 and 4.12 (m, 2H, H_2-6'), 3.84 and 3.83 (two s, 6H, 2 x OCH_3), 3.75 (m, 1H, H-5'), 2.15, 2.05, 2.04, and 1.99 (four s, 12H, 4 x CH_3CO). ^{13}C NMR: δ 170.3, 170.2, 170.1 and 169.5 (4 x CO), 154.7 (C-2), 152.2 (C-5), 122.3 (C-3), 115.1 (C-6), 111.9 (C-1), 98.8 (C-1'), 88.5 (C-4), 87.3 and 82.9 (C≡C), 70.9 (C-3'), 70.8 (C-5'), 70.7 (C-2'), 68.6 (C-4'), 68.5 (C-6'), 61.36 ($\underline{C}H_2C\equiv$), 57.1, 57.0, and 56.5 (2 x OCH_3), 20.7 and 20.6 (4 x $\underline{C}H_3CO$). Anal. Calcd for $C_{25}H_{29}IO_{12}$ (648.40): C, 46.31; H, 4.51. Found: C, 46.44; H, 4.50.

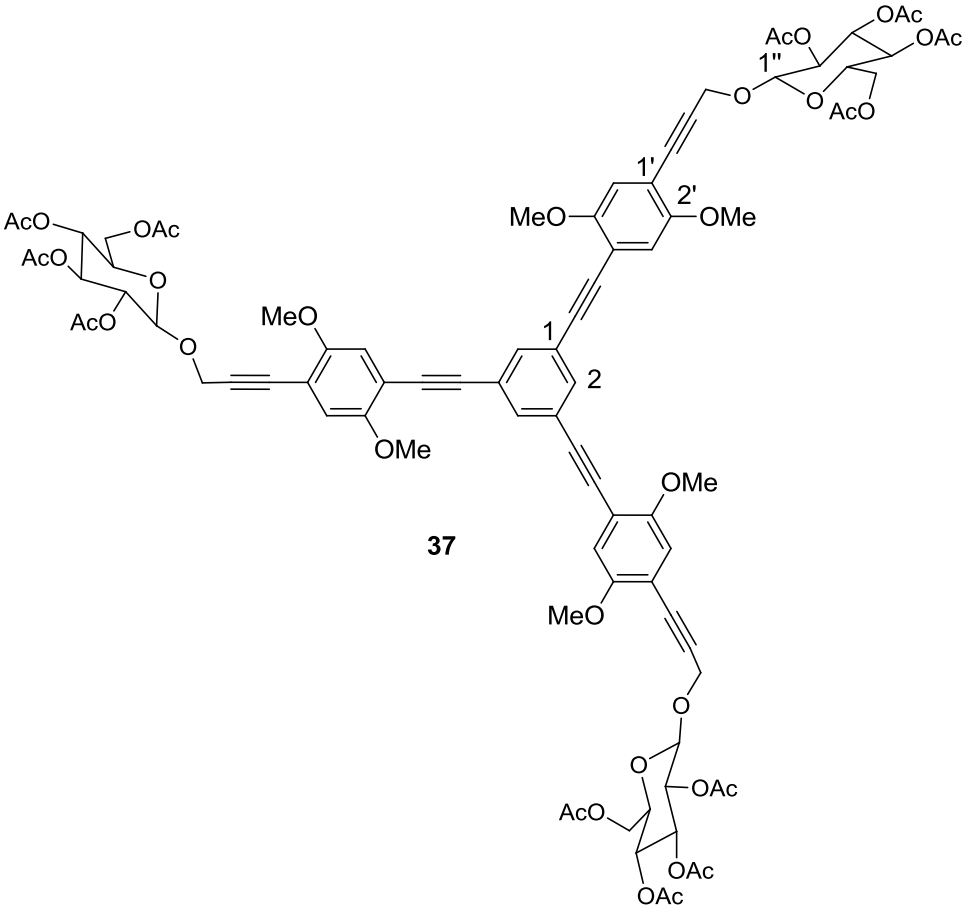
Compound 35. **16** (0.22 g, 0.61 mmol, 1 equiv), **34** (0.75 g, 1.16 mmol, 2 equiv), $Pd(PPh_3)_4$ (0.10 g, 0.09 mmol, 0.15 equiv), Ag_2O (0.27 g, 1.16 mmol, 2 equiv) were suspended in dry DMF (4 mL) and THF (2 mL). The mixture was heated at 70 °C for 30h and maintained under Ar atmosphere and continuous stirring until the disappearance of starting products. After filtration over Celite, the solvents were removed under reduced pressure, and the obtained reaction crude was subjected to silica gel column chromatography with hexane/EtOAc 50:50 as eluant, and compound **35** was obtained as a yellow solid (0.31 g, 0.25 mmol, 41%).



35

TLC: R_f 0.36 (hexane/EtOAc 40:60). Mp 181°-183°.^{1}H NMR: δ 7.48 (d, $J_{5,6}$ = 8.4, 1H, H-6), 7.09 (m, 2H, H-3,5), 7.00, 6.99, 6.93 and 6.91 (four s, 4H, 2 x H-3',6'), 5.42 (d, 2H, $J_{3'',4''}$ = 2.0, 1H, H-4''), 5.26 (dd, 2H, $J_{2'',3''} = J_{1'',2''}$ = 8.0, $J_{2'',4''}$ = 2.0, 1H, H-2''), 5.09 (dd, 2H, $J_{3'',4''} = J_{3'',2''}$ = 7.0, $J_{1'',3''}$ = 3.0, 1H, H-3''), 4.86 (d, 2H, $J_{1'',2''}$ = 8.0, 1H, H-1''), 4.67 (s, 4H, 2x $CH_2C{\equiv}$), 4.27 and 4.18 (m, 4H, H_2-6''), 3.89 and 3.87 (three s, 12H, 4 x OCH_3), 3.75 (m, 2H, H-5''), 3.04 [*broad* s, 6H, $N(CH_3)_2$], 2.16, 2.07, 2.04, and 2.00 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.4, 170.2, 170.1, and 169.6 (8 x CO), 154.1and 153.8 (C-2, 2 x C-2',5'), 134.4 (C-6), 128.8 (C-4), 123.8 and 120.0 (C-3,5), 115.7, 115.6 and 115.1 (2 x C-3',6'), 113.8, 112.3 and 111.9 (C-1, 2 x C-1',4'), 98.9 (2 x C-1"), 90.7, 89.2, 86.3, 83.5 (4 x C≡C), 70.9 (2 x C-3"), 70.8 (2 x C-5"), 68.7 (2 x C-2"), 67.0 (2 x C-4"), 61.2 (2 x C-6"), 57.1 (2 x $\underline{C}H_2C{\equiv}$), 56.4 and 56.3 (4 x OCH_3), 43.5 [$N(CH_3)_2$], 20.8, 20.7 and 20.6 (8 x $\underline{C}H_3CO$).

Compound 37. To commercial 1,3,5 ethynyl benzene (0.20 g, 1.3 mmol, 1 equiv), **4** (2.59 g, 4 mmol, 3 equiv) and $Pd(PPh_3)_4$ (0.45 g, 0.39 mmol, 0.30 equiv) in dry DMF (20 mL) was slowly added Et_3N (20 mL). The solution under inert atmosphere was heated at 60 °C and maintained under continuous stirring for 4h until the disappearance of **4** by (hexane/EtOAc 4:6). Column chromatography was performed with Hexane/AcOEt 50:50 (gradient to Hexane/AcOEt 10/90) as eluant, and compound **37** was obtained as a white solid (0.90 g, 0.52 mmol, 40%).

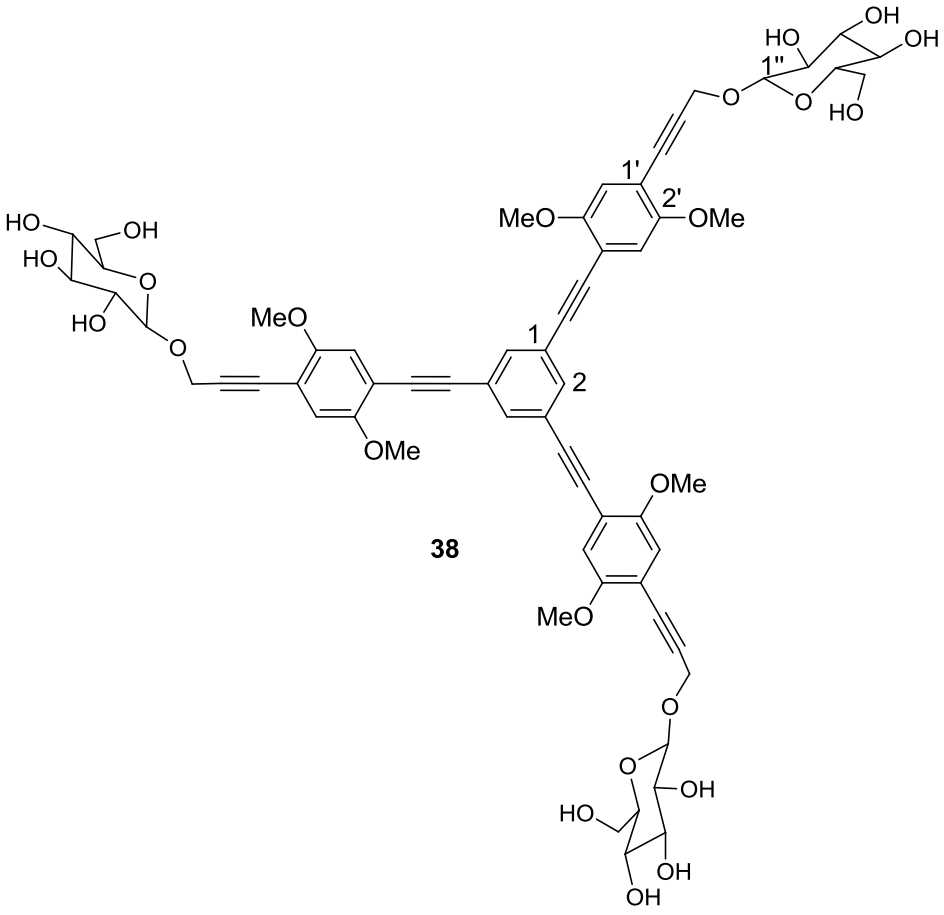


37

TLC: R_f 0.64 (hexane/EtOAc 70:30). Mp 65-67 °C. ^{1}H NMR: δ 7.71 (s, 3H, H-2, H-4, H-6), 7.00 and 6.84 (2s, 6H, 3xH-3', 3xH-6'), 5.26 (t, $J_{2",3"} = J_{3",4"}$ = 9.3, 3H, 3 x H-3"), 5.12 (t, $J_{3",4"} = J_{4",5"}$ = 9.3, 3H, 3x H-4"), 5.04 (dd, $J_{1",2"}$ = 8.3, $J_{2",3"}$ =9.3, 3H, 3x H-2"), 4.91 (d, $J_{1",2"}$ = 8.3, 3H, 3x H-1"), 4.65 (s, 6H, 3x $CH_2C\equiv$), 4.30 and 4.15 (split AB system, $J_{5",6"A}$ = 4.9, $J_{5",6"B}$ = 2.4, $J_{6"A,6"B}$ = 12.2, 6H, 3 x H_2-6"), 3.89 and 3.88 (two s, 24H, 8 x OCH_3), 3.76 (ddd, $J_{4",5"}$ = 9.3, $J_{5",6"A}$ = 4.9, $J_{5",6"B}$ = 2.4, 3H, 3 x H-5"), 2.07, 2.04, 2.02, and 2.01 (four s, 36H, 12 x CH_3CO). ^{13}C NMR: δ 170.4, 170.0, 169.2, and 169.2 (12 x CO), 153.9 and 153.8 (3 x C-2',5'), 134.1 (C-2,3,5,6),

123.7 (C-1,4), 115.6 and 115.4 (3 x C-3',6'), 113.2 and 112.4 (3 x C-1',4'), 98.2 (3 x C-1"), 98.1, 93.2, 89.1, and 83.2 (6 x C≡C), 72.6 (3 x C-3"), 71.7 (3 x C-5"), 71.0 (3 x C-2"), 68.2 (3 x C-4"), 61.6 (3 x C-6"), 56.9 (3 x $\underline{C}H_2C\equiv$), 56.3, 56.2, 56.1 and 56.0 (6 x OCH_3), 20.5 and 20.4 (12 x $\underline{C}H_3CO$).

Compound 38. It was obtained following **procedure A** starting from **37** (0.20g, 0.12mmol) with the final obtaining of compound **36** as a brilliant yellow solid (0.13g, 0.11mmol, 94%).



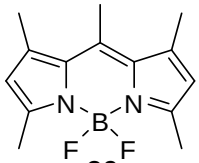
38

TLC: *Rf*0.05 ($CHCl_3$/MeOH 80:20). Mp 123-125°C. ^{1}H NMR: 7.68 (s, 3H, H-2, H-4, H-6), 7.23 and 7.10 (2s, 6H, 3xH-3', 3xH-6'), 5.14 (d, J_{vic} = 4.9, 3H, 3 x OH), 4.97 (d, J_{vic} = 4.9, 3H, 3 x OH), 4.93 (d, J_{vic} = 5.4, 3H, 3 x OH) , 4.69 and 4.52 (AB system, J_{gem} = 15.7, 4H, 3 x $CH_2C\equiv$), 4.53 (t, $J_{OH,6}$= 4.9, 3H, 3 x 6'-OH), 4.33 (d, $J_{1',2'}$ = 7.9, 3H, 3 x H-1'), 3.84 and 3.81 (s, 9H, 3 x

OCH_3), 3.69 and 3.67 (split AB m, 6H, 3 x H_2-6'), 3.16 - 3.00 (m, 12H, 3 x H-2'-5'). ^{13}C NMR (dmso-*d6*): δ 153.7 and 153.6 (C-2,5), 115.9 (C-3,6), 112.8 and 111.9 (C-1,4), 101.1 (3 x C-1'), 91.5, 87.7 and 82.0 (3 x C≡C), 77.0 and 76.7 (3 x C-3',5'), 73.3 (3 x C-2'), 70.1 (3 x C-4'), 61.2 (3 x C-6'), 56.3, 56.2 (3 x OCH_3), 55.9 (3 x $\underline{C}H_2C\equiv$).

Compound 39[4]: First step**:** To a solution of 2,4-dimethylpirrole (2.42g, 25.5 mmol, 1eq.) in dry CH_2Cl_2 (80 mL), at RT and under Ar atmosphere, was slowly added AcCl (0.91mL, 12.75 mmol, 0.5eq.). the mixture was heated at 50° for 3 hours under stirring. The solvent was removed under pressure to give a red solid.

Second step: The red solid was suspended in a mixture 2:1 Toluene/CH_2Cl_2 (160/8mL), after adding of triethylamine (8.51 mL, 61,15mmol,) the suspension was maintained at RT and under stirring until the complete solubilization (30 minutes). To the solution were added BF_3OEt_2 (10.85 mL, 87.91 mmol,) and the mixture was heated at 60° for 1,5 h. The volatiles were removed under reduced pression and the crude purified by column chromatography on silica gel using Hexane/EtOAc (90:10) as eluant to give compound **39** as a brilliant red solid (2.68g, 42%). TLC: *Rf*0.35. Mp.= 158-160°C. *Rf* = 0.75 (hexane/EtOAc 8.2).



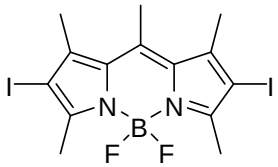
39

Compound 40[5]: 2.75 g of N-iodosuccinimide (12.21 mmol) were dissolved in dry CH_2Cl_2 (35 mL) and were slowly added to a solution of **39** (1,60 g, 6.10 mmol) in CH_2Cl_2 (20 mL). The mixture was maintained at RT overnight. The solvent was evaporated *in vacuo* and the crude

[4] Florian, A.; Mayoral, M. J.; Stepanenko, V.; Fernandez, G. *Chem. Eur. J.* **2012**, *18*, 14957-14961

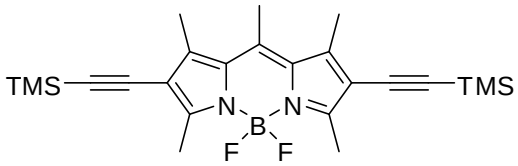
[5] Bonnier, C.; Machin, D. D.; Abdi, O.; Koivisto, B. D. *Org Biomol Chem.* **2013**, *11*, 3756-3760

purified by column chromatography on silica gel using Hexane/EtOAc (from 90:10 to 70/30) as eluant to give compound **40** as a brilliant red solid (2.5g, 80%). Mp. 117-119°C. *Rf* = 0.65 TLC (hexane/AcOEt 8:2).



40

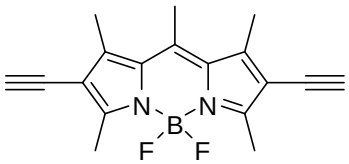
Compound 41. 40 (1.10g, 2.14 mmol), $Pd(PPh_3)Cl_2$ (57 mg, 0.08 mmol) and CuI (16 mg, 0.08 mmol) were dissolved in dry THF (35 mL). Then dry Et_3N (35 mL) and ethyniltrimthylsilane (1.82 mL, 12.84 mmol) were added and the mixture was heated at 70°C for 20h. The volatiles were removed under reduced pression and the crude purified by column chromatography on silica gel using Hexane/CH_2Cl_2 (1:2) as eluant to give compound **41** as a orange solid (0.700 g, 71%). Mp 129-131°C. *Rf* = 0.50 (TLC hexane/CH_2Cl_2 1:1).



41

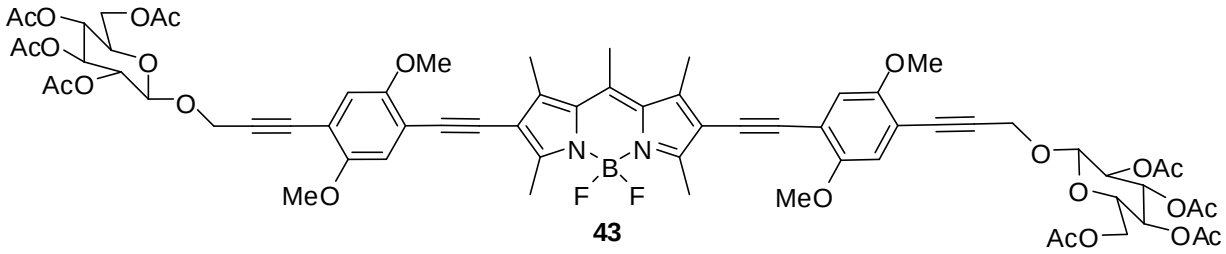
Compound 42.[6] 41 (0.60 g, 1.93 mmol) was dissolved in dry THF (20 mL). To the mixture, cooled at -78°C and under vigorous stirring and under Ar atmosphere, TBAF in THF (5.7mL, 1M,) was added dropwise. The solution was maintained at RT for 5h. By adding water it is formed a precipitate that was collected by filtration. **42** was obtained as a dark red solid (0.300g, 73%). Mp 114-116°C. *Rf* = 0.40 (Hexane/EtOAc 8:2).

[6] Lin, H.-Y.; Huang, W.-C.; Chen, Y.-C.; Chou, H.-H.; Hsu, C.-Y.; Lin, J. T.; Lin, H.-W. *Chem. Commun.* **2012**, *48*, 8913–8915



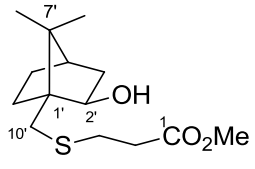
42

Compound 43. Compound **4** (0.83 g, 1.29 mmol, 2.0 equiv) and **42** (0.20 g, 0.65 mmol, 1 equiv) were dissolved in dry DMF (10 mL). To this mixture was added $Pd(PPh_3)_4$ (0.09 g, 0.08 mmol, 0.12 equiv) and then (10 mL) Et_3N. The mixture, under Ar atmosphere, was heated at 65 °C and maintained under continuous stirring for 12 h until the disappearance of **4** by (hexane/EtOAc 2:8). Column chromatography was performed with hexane/EtOAc 50:50 (gradient to hexane/AcOEt 30:70) as eluant, and compound **43** was obtained as a violet oil (0.30 g, 0.22 mmol, 34%). TLC: R_f 0.35 (EtOAc/hexane 70: 30).^{1}H-NMR of impure product is reported in figure 1 (Chapter 3).



43

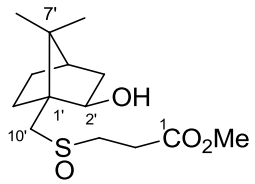
Compound 45. To a solution of thiol **44**(1.90 g, 10.20 mmol) in anh. THF (43 ml), under Ar atmosphere at –78°, Triton B sol. (40 wt.% in MeOH, 0.51 ml, 1.33 mmol) was added. After 5 min stirring at -78°, methyl acrylate (1.03 ml, 11.44 mmol) was added and the reaction was allowed to reach r.t. and left stirring until completion (overnight). The mixture was concentrated *in vacuo* and the crude residue was purified by flash cc (AcOEt / hexane 1:9) to provide sulfide **45** (2.63 g, 9.65 mmol, 95%) as a transparent oil.



45

R_f 0.65 (AcOEt / hexane 3:7). ^{1}H-NMR ($CDCl_3$): 3.85 (*dd*, $J_{2',3'}$ = 8.3, 1H, H-2'), 3.70 (*s*, 3H, OCH_3), 2.63 and 2. 84 (*m*, 7H, H_2-2, H_2-3, H_2-10', OH), 1.00 and 1.76 (*m*, 7H, H_2-3', H-4', H_2-5', H_2-6'), 0.82 and 1.05 (2 *s*, 6H, H_3-8', H_3-9'). ^{13}C-NMR ($CDCl_3$): 172.5 (C-1) , 76.7 (C-2'), 52.1 (C-1') , 51.9 (OCH_3), 47.6 (C-7') , 45.1 (C-4') , 39.1 (C-3'), 34.2 (C-2) , 31.9 (C-10') , 28.4 (C-3), 27.1 and 31.0 (C-5'and C-6')19.9 and 20.6 (C-8'and C-9'). Anal. calc. for $C_{14}H_{24}O_3S$ (272.40): C 61.73, H 8.88; found: C 61.71, H 8.89.

Compound 46 (epimeric mixture of sulfoxides). A sol. of **45** (2.61 g, 9.58 mmol) in CH_2Cl_2 (40 ml) was stirred at –78° and a sol. of *m*-CPBA (77%, 2.15 g, 9.58 mmol) in CH_2Cl_2 (40 ml) was slowly added. The reaction was monitored by (hexane/EtOAc 8:2) and appeared complete by the end of the oxidant addition. The reaction was quenched by adding aq. $Na_2S_2O_3$ (10 wt.%) and the combined organics were washed twice with a satd. $NaHCO_3$ sol. and twice with brine, dried (Na_2SO_4), filtered, concentrated *in vacuo* to provide sulfoxide **46** (2.62 g, 9.08 mmol, 95%) as a transparent oil. Major epimer R_f 0.10 (hexane/EtOAc 7:3).

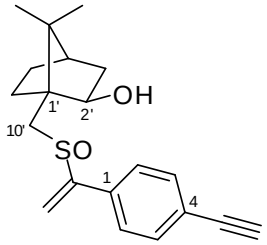


46

^{1}H-NMR ($CDCl_3$): 4.02 (*dd*, $J_{2',3'}$ = 8.3, 3.9, H-2'), 3.71 (*s*, 3H, OCH_3), 0.63 and 1.00 (2*s*, 6H, H_3-8', H_3-9'), 2.79-3.11 (*m*, 5H, H_2-2, H_2-3, OH) 2.38 and 3.25 (*AB* system, 2H, $J_{10'A,10'B}$ = 13.2, H_2-10'),1.05-1.85 (*m*, 7H, H_2-3', H-4', H_2-5', H_2-6'). ^{13}C-NMR ($CDCl_3$): 171.6 (C-1), 76.8 (C-2'), 53.1 (C-10'), 52.2 (OCH_3), 49.2 (C-1'), 48.1 (C-7'), 48.0 (C-3), 44.9 (C-4'), 38.4 (C-3'), 30.8 (C-5'), 27.0 (C-6'), 26.7 (C-2), 19.8 (C-8'), 20.4, (C-9'). Anal. calc. for $C_{14}H_{24}O_4S$ (288.40): C 58.30, H 8.39; found: C 58.28, H 8.40.

General procedure of thermolysis of sulfoxides 46 in the presence of diethynylbenzenes:

A sol. of sulfoxides **46** (0.70 g, 2.43 mmol) and commercial diethynyl acceptor (1.84 g, 14.59 mmol) in DCE (10 ml) was heated at reflux (83 °C) and maintained under stirring until the disappearance of starting sulfoxides by (hexane/EtOAc 8:2) (overnight). The solvent was removed under reduced pressure and the crude material was purified by cc on silica gel (hexane/ AcOEt, gradient from 9:1 to 7:3) to give the unreacted alkyne and **48** (70% total yield of epimeric mixture) or **49** (75% total yield of epimeric mixture). Epimers are reported in order of retention time.

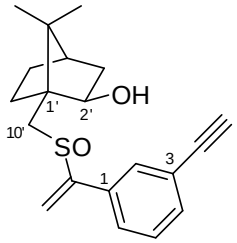


48_R/48_S

Compound 48R. (major, more mobile, epimer). White crystals, yield 60%, M.p. 164-166°C, R_f 0.75 (hexane /AcOEt 7: 3).^{1}H-NMR ($CDCl_3$): 7.35 and 7.53 (*AA'BB'* system, J = 8.3, 4H, H-2, H-3, H-5, H-6), 6.07 and 6.14 (2 *s*, 2H, $=CH_2$), 4.16 (*dd*, $J_{2',3'}$ = 7.9 , 4.4, 1H, H-2'), 2.35 and 2.95 (*AB* system, $J_{10'A,10'B}$ = 13.7, 2H, H_2-10'), 3.17 (*s*, 1H, H-C≡), 1.05 and 1.85 (*m*, 7H, H_2-3', H-4', H_2-5', H_2-6'), 0.63 and 1.00 (2 *s*, 6H, H_3-8', H_3-9'). ^{13}C-NMR ($CDCl_3$): 152.3 (C=), 134.0 (C-1), 126.2 and 132.9 (C-2, C-3, C-5, C-6), 123.4 (C-4), 117.5 ($=CH_2$), , 79.1 (≡CH), 82.7 (C≡), 76.9 (C-2'), 55.2 (C-10'), 51.5 (C-1'), 48.1 (C-7'), 45.1 (C-4'), 38.4 (C-3'), 27.1 and 30.8 (C-5', C-6'), 19.8 and 20.3 (C-8', C-9'). Anal. calc. for $C_{20}H_{24}O_2S$ (328.47): C 73.13, H 7.36; found: C 73.16, H 7.37.

Compound 48S. (minor, less mobile, epimer). Pale yellow oil, yield 10%, R_f 0.45 (hexane / AcOEt 7: 3). ^{1}H-NMR ($CDCl_3$): 7.39 and 7.50 (*AA'BB'* system, J_{ortho} = 8.3, 4H, H-2, H-3, H-5, H-6) 6.09 and 6.16 (2 *s*, 2H, $=CH_2$), 4.10 (*dd*, $J_{2',3'}$ = 7.8, 3.9, 2H, H-2'), 3.38 (*broad* s, 1H, OH), 3.16 (*s*, 1H, ≡CH), 2.37 and 3.21 (*AB* system, $J_{10'A,10'B}$ = 14.1, 2H, H_2-10), 1.05-1.80 (*m*, 7H, H_2-

3', H-4', H_2-5', H_2-6'), 0.65 and 0.99 (2 *s*, H_3-8', H_3-9'). ^{13}C-NMR ($CDCl_3$): 151.9 (C=), 134.1 (C-1), 126.3 and 132.7 (C-2, C-3, C-5, C-6), 123.2 (C-4), 118.2 (=CH_2), 82.7 (C≡),79.0 (≡CH), 76.2 (C-2'), 52.3 and 52.4 (C-1'and C-10'), 48.9 (C-7'), 44.5 (C-4'), 40.0 (C-3'), 29.6 and 31.3 (C-5', C-6'), 20.1 and 20.3 (C-8', C-9'). Anal. calc. for $C_{20}H_{24}O_2S$ (328.47): C 73.13, H 7.36; found: C 73.11, H 7.35.



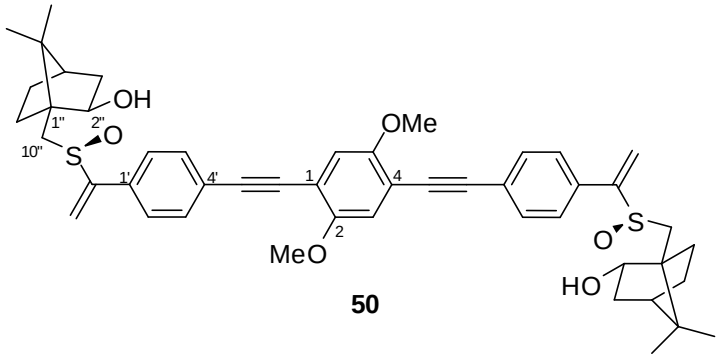
49_R/49_S

Compound 49R (major, more mobile, epimer). Yellow crystals, yield 64%, Mp 108-110°C, *R*f 0.78 (hexane /AcOEt 7: 3). ^{1}H-NMR ($CDCl_3$): 7.36 and 7.54 (*m*, 4H, H-2, H-4, H-5, H-6), 4.17 (*dd*, $J_{2',3'}$ = 8.3, 3.9, 2H, H-2'), 6.07 and 6.14 (2 *s*, 2H, =CH_2), 3.94 (*broad* s, 1H, OH), 3.15 (*s*, 1H, ≡CH), 2.36 and 2.96 (*AB* system, $J_{10'A,10'B}$ = 13.2, 2H, H_2-10'), 1.10 and 1.93 (*m*, 7H, H_2-3', H-4', H_2-5', H_2-6'), 0.64 and 1.02 (2 *s*, 6H, H_3-8', H_3-9'). ^{13}C-NMR ($CDCl_3$): 152.0 (C=) 133.7 (C-1), 126.4, 129.1, 129.7 and 132.8 (C-2, C-4, C-5, C-6), 123.2 (C-3), 117.4 (=CH_2), 82.3 (C≡),78.5 (≡CH), 76.7 (C-2'), 54.9 (C-10'), 51.3 (C-1'), 47.9 (C-7'), 44.9 (C-4'), 38.2 (C-3'), 26.9 and 30.6 (C-5', C-6'), 19.8 and 20.2 (C-8', C-9'). Anal. calc. for $C_{20}H_{24}O_2S$ (328.47): C 73.13, H 7.36; found: C 73.15, H 7.36.

Compound 49S (minor, less mobile, epimer). Pale yellow oil, yield 11%, *R*f 0.50 (hexane /AcOEt 3:7). ^{1}H-NMR ($CDCl_3$): 7.32-7.53 (*m*, 4H, H-2, H-4, H-5, H-6), 6.05 and 6.12 (2 *s*, 2H, =CH_2), 4.07 (*dd*, $J_{2',3'}$ = 7.5, 3.5, 2H, H-2'), 3.12 (*s*, 1H, ≡CH), 3.11 (*broad s*, 1H, OH), 2.33 and 3.17 (*AB* system, $J_{10'A,10'B}$ = 14.2, 2H, H_2-10'), 1.00 and 1.83 (*m*, 7H, H_2-3', H-4', H_2-5', H_2-6'), 0.62 and 0.96 (2 *s*, 6H, H_3-8', H_3-9'). ^{13}C-NMR ($CDCl_3$): 151.5 (C=), 134.1 (C-1), 126.7, 129.0,

129.8 and 132.7 (C-2, C-4, C-5, C-6), 123.0 (C-3), 118.0 (=CH_2), 82.6 (C≡),78.4 (≡CH), 76.1 (C-2'), 52.2 and 52.5 (C-1', C-10'), 48.7 (C-7'), 44.5 (C-4'), 40.1 (C-3'), 27.3 and 31.2 (C-5'), C-6'), 20.0 and 20.2 (C-8', C-9'). Anal. calc. for $C_{20}H_{24}O_2S$ (328.47): C 73.13, H 7.36, found: C 73.14, H 7.35.

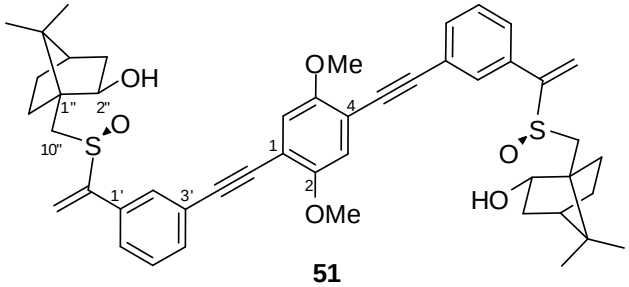
Compound 50 and 51. $Pd(PPh_3)_4$ (0.07 g, 0.06 mmol), 1,4-diiodo-2,5-dimethoxybenzene (0.20 g, 0.51 mmol) and the alkyne **48R** or **49R** (0.39 g, 1.19 mmol) were dissolved in dry DMF (8 ml). To this mixture Et_3N (8 ml) was added. The mixture was heated at 60 °C and maintained under continuous stirring under Ar until the disappearance of the alkyne was verified by (hexane/EtOAc 1:1) Solvents were removed under reduced pressure. The reaction crude was subjected to silica gel cc (eluant hexane / AcOEt 3:7).



50

Compound 50. 3 Hours reaction time, Yellow crystals, yield 85%, M.p. 200-202°C, *R*f 0.55 (hexane /AcOEt 6:4). ^{1}H-NMR ($CDCl_3$): 7.40 and 7.62 (*AA'BB'* system, J_{ortho} = 8.3, 4H, H-2",H-3", H-5", H-6"), 7.04 (*s*, 2H, H-3, H-6), 6.10 and 6.15 (2 *s*, 2H, =CH_2), 4.17 (*dd*, $J_{2',3'}$ = 8.3, 4.4, 1H, H-2'), 3.94 (*broad s*, 1H, OH), 3.92 (*s*, 3H, OCH_3), 2.38 and 2.97 (*AB* system, $J_{10'A,10'B}$ = 13.4, 2H, H_2-10'), 1.88 and 1.11 (*m*, H_2-3', H-4', H_2-5', H_2-6'), 0.64 and 1.02 (2 *s*, H_3-8', H_3-9'). ^{13}C-NMR ($CDCl_3$): 153.9 (C-2), 152.3 (C=), 132.0 (C-1", C-5), 126.2, 132.4 (C-2", C-3", C-5", C-6"),124.4 (C-4"),117.2 (=CH_2), 115.5 (C-3, C-6), 113.2 (C-1, C-4), 92.2 and 87.4 (C≡C), 76.9 (C-2'), 56.4 (OCH_3), 55.2 (C-10'), 51.4 (C-1'), 48.0 (C-7'), 45.0 (C-4'), 38.4 (C-3'), 30.8 and 27.0

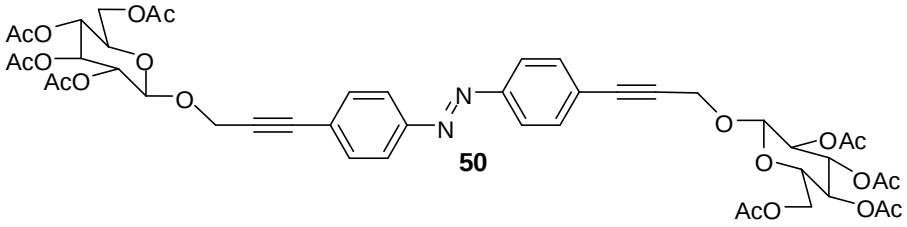
(C-5', C-6'), 20.3 and 19.7 (C-8', C-9'). Anal. calc. for $C_{48}H_{54}O_6S_2$ (791.07): C 72.88, H 6.88; found: C 72.90, H 6.90.



51

Compound 51. 20 Hours reaction time, Yellow crystals, yield 80%, Mp 102-104°C; *R*f 0.47 (hexane /AcOEt 6: 4). ^{1}H-NMR ($CDCl_3$): 7.61 and 7.34 (*m*, 3H, H-2'', H-4'', H-6''), 7.06 (*s*, 2H, H-3, H-6), 6.09 and 6.16 (2 *s*, 2H, $=CH_2$), 4.17 (*ddd*, $J_{2',3'}$ = 7.8, 3.9, $J_{2',OH}$ = 2.9, 1H, H-2'), 3.95 (*d*, 1H, OH), 3.93 (*s*, 3H, OCH_3), 2.39 and 2.98 (*AB* system, $J_{10'A,10'B}$ = 13.2, H_2-10'), 1.12-1.83 (*m*, 7H, H_2-3', H-4', H_2-5', H_2-6'), 0.62 and 1.02 (2 *s*, H_3-8', H_3-9'). ^{13}C-NMR ($CDCl_3$): 153.9 (C-2, C-5), 152.3 (C=), 134.0 (C-1''), 126.1, 129.2, 129.6 and 132.5 (C-2'', C-4'', C-5'', C-6''), 124.4 (C-3''), 117.6 ($=CH_2$), 115.5 (C-3, C-6), 113.2 (C-1, C-4), 86.7 and 94.0 (C≡C), 76.9 (C-2'), 56.4 (OCH_3), 55.1 (C-10'), 51.4 (C-1'), 48.1 (C-7'), 45.1 (C-4'), 38.4 (C-3'), 27.0 and 30.7 (C-5', C-6'), 19.7 and 20.3 (C-8', C-9'). Anal. calc. for $C_{48}H_{54}O_6S_2$ (791.07): C 72.88, H 6.88; found: C 72.91, H 6.87.

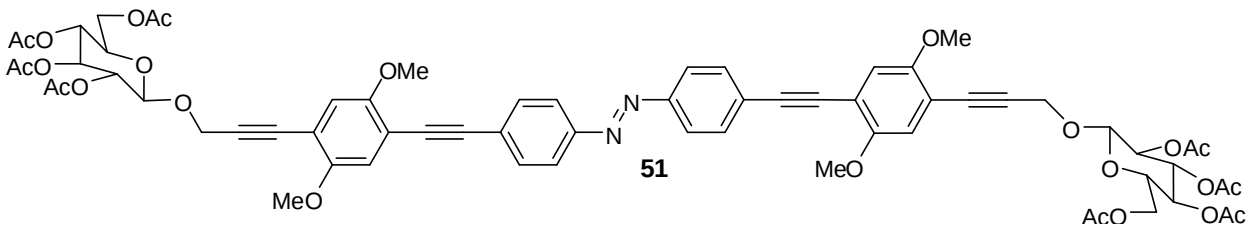
Compound 50. Azobenzene **49** (1.00 g, 2.30 mmol, 1 equiv), **1** (1.66 g, 4.60 mmol, 2 equiv), $Pd(PPh_3)_4$ (0.32 g, 0.28 mmol, 0.12 equiv) were dissolved in dry DMF (10 mL). To the mixture was added slowly Et_3N (10 mL). The mixture under Ar atmosphere was heated at 60 °C and maintained under continuous stirring. The reaction was monitored by TLC (hexane/EtOAc 6:4) and appeared complete by the end of the compound **1**. The volatiles were removed *in vacuo*. Column chromatography was performed with hexane/AcOEt 70:30 as eluant, and compound **50** was obtained as a dark orange solid (2.50 g, 2.63mmol, 81%). TLC: R_f 0.40 (hexane/EtOAc 60:40).



50

^{1}H NMR: δ 7.90 (d, $J_{2,3} = J_{5,6} = 8.7$, 4H, H-3,5), 7.59 (d, $J_{2,3} = J_{5,6} = 8.7$, 4H, H-2,6), 5.28 (t, $J_{2',3'} = J_{3',4'} = 9.3$, 2H, 2 x H-3'), 5.14 (t, $J_{3',4'} = J_{4',5'} = 9.3$, 2H, 2 x H-4'), 5.06 (dd, $J_{1',2'} = 8.3$, $J_{2',3'} = 9.3$, 2H, 2 x H-2'), 4.86 (d, $J_{1',2'} = 8.3$, 2H, 2 x H-1'), 4.64 (s, 4H, 2 x $CH_2C\equiv$), 4.32 and 4.15 (split AB system, $J_{5',6'A} = 5.6$, $J_{5',6'B} = 2.4$, $J_{6'A,6'B} = 12.2$, 4H, 2 x H_2-6'), 3.77 (ddd, $J_{4',5'} = 9.3$, $J_{5',6'A} = 5.6$, $J_{5',6'B} = 2.4$, 2H, 2 x H-5'), 2.09, 2.06, 2.03, and 2.02 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.7, 170.3, 169.5, and 169.4 (8 x CO), 152.0 (2xC-1), 132.6 (2xC-2,6), 123.1 (2xC-3,5), 98.5 (2 x C-1'), 89.7 and 86.1 (2 x C≡C), 72.8 (2 x C-3'), 71.9 (2 x C-5'), 71.1 (2 x C-2'), 68.3 (2 x C-4'), 61.8 (2 x C-6'), 57.0 (2 x $\underline{C}H_2C\equiv$), 20.7 and 20.6 (8 x $\underline{C}H_3CO$).

Compound 51. Azobenzene **49** (1.00 g, 2.30 mmol, 1 equiv), **4** (2.95 g, 4.6 mmol, 2 equiv), $Pd(PPh_3)_4$ (0.32 g, 0.27 mmol, 0.12 equiv) and Ag_2O (1.00g, 4.6mmol, 2equiv) were weighted in a flask. The solids were suspended in dry DMF/THF (2:1, 18mL). The obtained mixture under Ar atmosphere was heated at 60 °C and maintained under continuous stirring for 7h until the disappearance of **4** by TLC (hexane/EtOAc 6:4). The solution was filtered on Celite/Silica 1:1 and the volatiles were removed *in vacuo*. Column chromatography was performed with hexane/AcOEt 50:50 as eluant, and compound **51** was obtained as a brilliant orange solid (1.60 g, 1.26mmol, 54%). TLC: *R*f 0.25.



51

^{1}H NMR: δ 7.93 (d, $J_{2,3}$ = $J_{5,6}$ = 8.7, 4H, 2xH-3,5), 7.72 (d, $J_{2,3}$ = $J_{5,6}$ = 8.7, 4H, 2xH-2,6), 7.05 and 6.96 (two s, 4H, 2xH-3',5') 5.28 (t, $J_{2'',3''}$ = $J_{3'',4''}$ = 9.3, 2H, 2 xH-3''), 5.13 (t, $J_{3'',4''}$ = $J_{4'',5''}$ = 9.3, 2H, 2 x H-4''), 5.06 (dd, $J_{1'',2''}$ = 8.3, $J_{2'',3''}$ = 9.3, 2H, 2 x H-2''), 4.66 (d, $J_{1'',2''}$ = 8.3, 2H, 2 x H-1''), 4.64 (s, 4H, 2 x $CH_2C{\equiv}$), 4.32 and 4.15 (split AB system, $J_{5'',6''A}$ = 5.6, $J_{5'',6''B}$ = 2.4, $J_{6''A,6''B}$ = 12.2, 4H, 2 x H_2-6''), 3.91 and 3.90 (two s, 12H, 4 x OCH_3), 3.77 (ddd, $J_{4'',5''}$ = 9.3, $J_{5'',6''A}$ = 5.6, $J_{5'',6''B}$ = 2.4, 2H, 2 x H-5''), 2.09, 2.07, 2.04, and 2.02 (four s, 24H, 8 x CH_3CO). ^{13}C NMR: δ 170.7, 170.3 and 169.4 (8 x CO), 154.1 (2xC-2',5'), 151.9 (2xC-1), 132.6 (2xC-2,6), 123.0 (2xC-3,5), 116.1 and 115.7 (2x C-3',6'), 113.6 and 112.8 (2xC-1',4'), 98.3 (2 x C-1''), 94.9, 89.2, 88.2 and 83.4 (2 x C≡C), 72.8 (2 x C-3''), 71.9 (2 x C-5''), 71.1 (2 x C-2''), 68.4 (2 x C-4'), 61.8 (2 x C-6''), 57.0 (2 x $\underline{C}H_2C{\equiv}$), 56.5 and 56.4 (4 x OCH_3), 20.7 and 20.6 (8 x $\underline{C}H_3CO$).

6.2 Equipment and methods for the absorption spectroscopy and photophysical experiments

UV/Vis absorption spectra were taken on a Jasco V-560 spectrophotometer. For steady-state luminescence measurements, a Jobin Yvon-Spex Fluoromax 2 spectrofluorimeter was used, equipped with a Hamamatsu R3896 photomultiplier. The spectra were corrected for photomultiplier response using software purchased with the fluorimeter. For the luminescence lifetimes, an Edinburgh OB 900 time-correlated single-photon-counting spectrometer was used. As excitation sources, a Hamamatsu PLP 2 laser diode (59 ps pulse width at 408 nm) and/or the nitrogen discharge (pulse width 2 ns at 337 nm) were employed. Emission quantum yields for acetonitrile deaerated solutions were determined using the optically diluted method.[3] As luminescence quantum yield standards we used an air equilibrated ethanol solution of anthracene (0.2).[4]

Experimental uncertainties on the absorption and photophysical data are as follows: absorption maxima, 2 nm; molar absorption, 15%; luminescence maxima, 4 nm; luminescence lifetimes, 10%; luminescence quantum yields, 20%.

Singlet oxygen evaluation. A direct comparison between the oligomers and Methylene blue species in solution by monitoring the UA photooxidation is not possible, because different excitation wavelengths were used for the photoproduction of $^1O_2$. Therefore, the efficiency of singlet-oxygen deliver was calculated by normalizing for the photon flux of the lamp at the lambda used for excitation (400 nm for OPEs and 600 nm for the methylene blue).

The absorption spectra were recorded in ultrapure spectroscopic solvents. UV/Vis absorption spectra were taken on a Jasco V-560 spectrophotometer. For steady-state luminescence measurements, a Jobin Yvon-Spex Fluoromax 2 spectrofluorimeter was used, equipped with a Hamamatsu R3896 photomultiplier. The spectra were corrected for photomultiplier response using software purchased with the fluorimeter. For the luminescence lifetimes, an Edinburgh OB 900 time-correlated single-photon-counting spectrometer was used. As excitation sources, a Hamamatsu PLP 2 laser diode (59 ps pulse width at 408 nm) and/or the nitrogen discharge (pulse width 2 ns at 337 nm) were employed. Emission quantum yields for acetonitrile deaerated solutions were determined using the optically diluted method.[37] As luminescence quantum yield standards we used an air equilibrated ethanol solution of anthracene (0.2).[38] Experimental uncertainties on the absorption and photophysical data are as follows: absorption maxima, 2 nm; molar absorption, 15%; luminescence maxima, 4 nm; luminescence lifetimes, 10%; luminescence quantum yields, 20%.

6.3 Biological study. Materials And Methods

Cell cultures. The human carcinoma cells used in this study were HEp-2 (derived from a larynx carcinoma) and HeLa (derived from a cervical carcinoma) cells.

HEp-2 cells were grown on culture slides and treated with compounds **9**, **10**, **11**, **18** and **31** to a final concentration of 100 μM (in RPMI medium) for 24h. The cells were subsequently analyzed using a DAPI filter (blue emission) or a FITC filter (green emission). In addition to evaluate the capability to emit also at lower concentrationHEp-2 cells were grown on 12 well plates and treated with a solution of compound-**20** to a final concentration of 100 μM, 10 μM and 1 μM (in RPMI medium) for 24h. The cells were subsequently detached from the substrate using trypsin and layered on polylysinated slides. Samples were analyzed using a DAPI filter (blue emission) (objectives 40 X) on a Biomed fluorescence microscope (Leitz, Wetzlar, Germany). To test the cells toxicity of compounds a standard trypan blue exclusion assay was employed.

The spontaneously transformed HaCaT cell line, obtained from human keratinocytes, has been used as control of nontumorogenic cells. HeLa and HaCaT cells were cultured using Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) fetal bovine serum (FBS), 50 units/mL penicillin and 50 μg/mL streptomycin. HEp-2 cells were cultured in RPMI medium also supplemented with FBS, penicillin and streptomycin (all from GE Healthcare Life Sciences, HyClone Laboratories, Logan, Utah, USA). Cell cultures were performed under standard conditions at 37ºC, 95% of humidity and 5% of CO_2 and the medium was changed each two days.

Photosensitizers administration. Stock solutions of both **18** (3-OPE18) and **28** (3-OPE28) were prepared in DMSO (Panreac) at a concentration of 10^{-3} M. The work solutions were obtained by dissolving the compounds in DMEM/RPMI with 1% FBS. The final concentration of DMSO was always lower than 0.5% (v/v), and the lack of toxicity of this solvent for the cells was also tested and confirmed. All the treatments were performed when cultures reached around 60-70% of confluence.

Intracellular localization of 18 (3-OPE18) and **28** (3-OPE28) For the analysis of **18** (3-OPE18) and **28** (3-OPE28) subcellular localization, the cells were plated on glass coverslips placed into wells and incubated with $1x10^{-6}$ M of **18** (3-OPE18) or **28** (3-OPE28) for 6 h at 37ºC. After incubation, cells were washed twice with PBS, mounted on slides with a drop of PBS and immediately observed under the fluorescence microscope coupled to a digital capture camera (Olympus BX61) using the appropriate filters of excitation light (UV, 365 nm, exciting filter UG-1). In addition, to better determine the intracellular localization of **1** (NMe_2) or **2** (bis-NMe_2), the distribution pattern of both compounds was compared with that of specific organelles. For this purpose, cells were incubated with known fluorescent probes for endoplasmic reticulum (ER-Tracker, Molecular Probes; Eugene, OR) and Golgi apparatus (NBD, Molecular Probes; Eugene, OR). The fluorescent probes were used at the concentrations indicated by the suppliers and were incubated for 30 min. After incubation, cells were washed twice in PBS and observed under fluorescence microscope. The fluorescence excitation and emission wavelengths of ER-Tracker were 374 and 430 nm respectively. In the case of NBD were 466 and 536 nm respectively.

Photodynamic treatments. Cells seeded on 24 well plates were incubated for 6 h with variable concentrations of **18 (3-OPE18) and 28 (3-OPE28)** (from 3 x 10^{-6} M to 2.5 x 10^{-5} M). Afterwards, the media were removing, replaced by fresh media and cells were irradiated with different UVA light doses, ranging from 0.25 to 2 J/cm^2 (UVA source: CAMAG, λ = 300-400 nm, power: 15.9 W/m^2 at 10 cm from the source). After irradiation, cells were washed three times with PBS, complete DMEM or RPMI was added, and were maintained in the incubator for 24 h until evaluation. To assess the possible dark cytotoxic effect of both PSs, cells were incubated in the dark for 18 h with different PS concentrations. In the same way, to test the effect of UVA light alone, cells were subjected to different light doses (0.25 - 2 J/cm^2).

Morphological Studies. Changes in general cell morphology and nuclear morphology after PDT were analyzed by phase contrast microscopy and Höescht-33258 staining, respectively. In the

second case, cells were fixed in formaldehyde (3.7% in PBS) for 15 min, washed and stained with 2.5 μg/mL Hoescht- 33258 dye (H-33258) (Sigma) for 5 min. After washing with distilled water, preparations were mounted with ProLong® Gold Antifade Reagent (Invitrogen). Death type was determinate analyzing nuclei morphology according to morphological criteria previously published.[39]

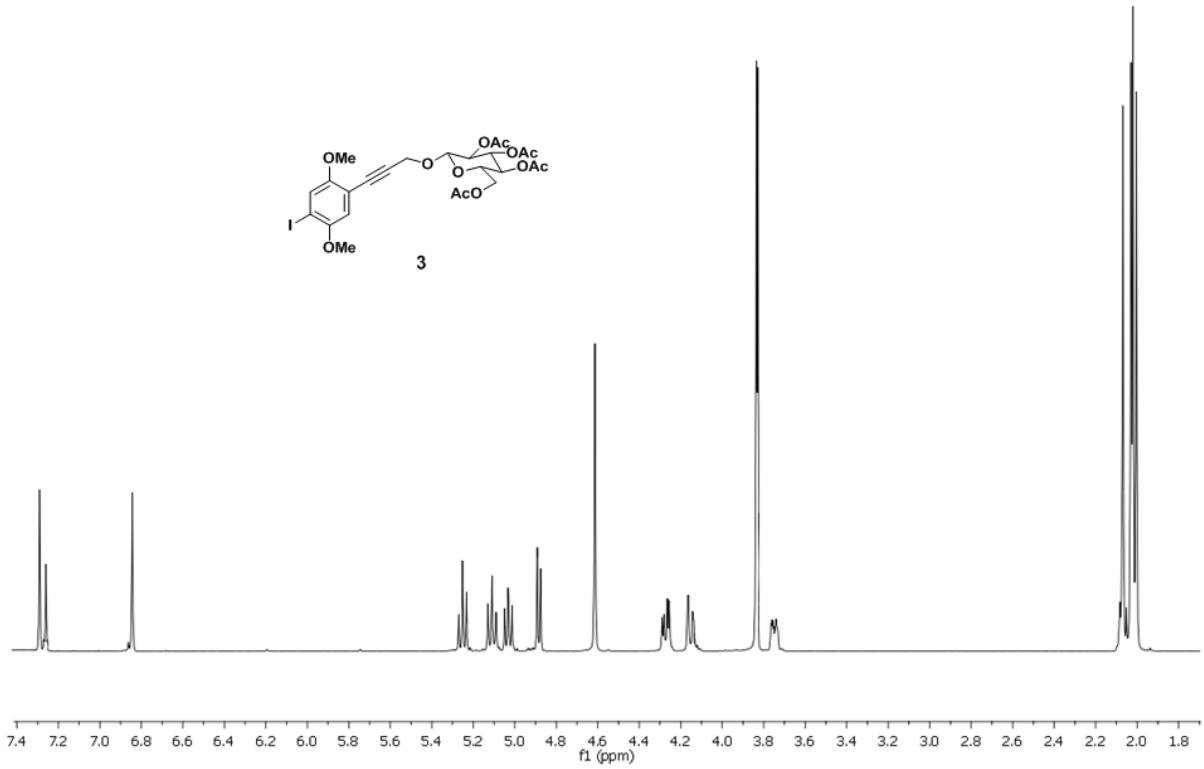
Viability assays. Cell viability was assessed 24 h after treatments using the MTT assay and the acridine orange (AO)/ethidium bromide (EtBr) method. Regarding the MTT assay, following treatments, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT Sigma, St Louis, USA) solution was added to each well at a final concentration of 50 μg/mL, and plates were incubated at 37˚C for 4 h. The formazan crystals were dissolved in DMSO and the absorbance at 542 nm was measured using a spectrophotometer (Espectra Fluor 4, Tecan). The results were expressed as cell survival percentage of control (cell survival (%) = (mean OD value of PDT-treated cells / mean OD value of control cells) x 100%).

The AO/EB method was applied according as previously described by Liu and cols.[40] This method allowed us to confirm the tendency of the cell death rate detected by MTT assay and, moreover, to determine approximately the type of cell death induced by PDT. AO is a vital dye able to stain nuclear DNA across an intact cell membrane, while EB can only stains cells that have lost their membrane integrity. A solution of PBS containing 50 μg/ml AO and 50 μg/ml EB was prepared. Then, 300 μL of this solution were added to each well and immediately analyzed under the fluorescent microscope. 200 cells of each condition were counted within 15 min after the addition of the fluorochromes. Cells were classified as (1) living cells (uniformly stained as green), (2) rounded cells (possible mitotic arrest and densely stained as green); (3) apoptotic cells (early apoptosis: densely stained as green but showing orange fragments; late apoptosis: densely stained as orange and condensed chromatin) and (4) necrotic cells (uniformly stained as orange-red stained and no chromatin condensation) (Figure S16, ESI).

Immunofluorescence. For immunodetection of α-tubulin, cells were raised on coverslips and subjected to PDT and, 24 h after treatment, were fixed and permeabilized in methanol (-20ºC) for 7 min. Then, cells were washed twice in PBS and incubated with α -tubulin primary antibody (Sigma-Aldrich. St. Louis, MO) for 1 h at 37ºC. After washing, cells were incubated with Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibody (1:250) (Invitrogen, California, USA) for 45 min at 37ºC, washed in PBS and mounted in Prolong Gold Antifade Reagent (Invitrogen, California, USA).

Microscopical observations and statistical analysis. Microscopic observation was carried out using a fluorescence microscope (Olympus BX61) equipped with the following filter sets for fluorescence microscopy: ultraviolet (UV, 365 nm, exciting filter UG-1), blue (450−490 nm, exciting filter BP 490), and green (545 nm, exciting filter BP 545). Images were obtained with the digital camera Olympus CCD DP70 and processed using the Adobe PhotoShop CS5 extended version 12.0 software (Adobe Systems Inc., USA).

Data are expressed as the mean value ± standard deviations (SD). Statistical analysis was carried out with SPSS Statistics 20.0 (IBM®). The statistical significance was determined using the T test for independent samples (*: $P<0.1$; **: $P<0.05$; ***: $P<0.01$).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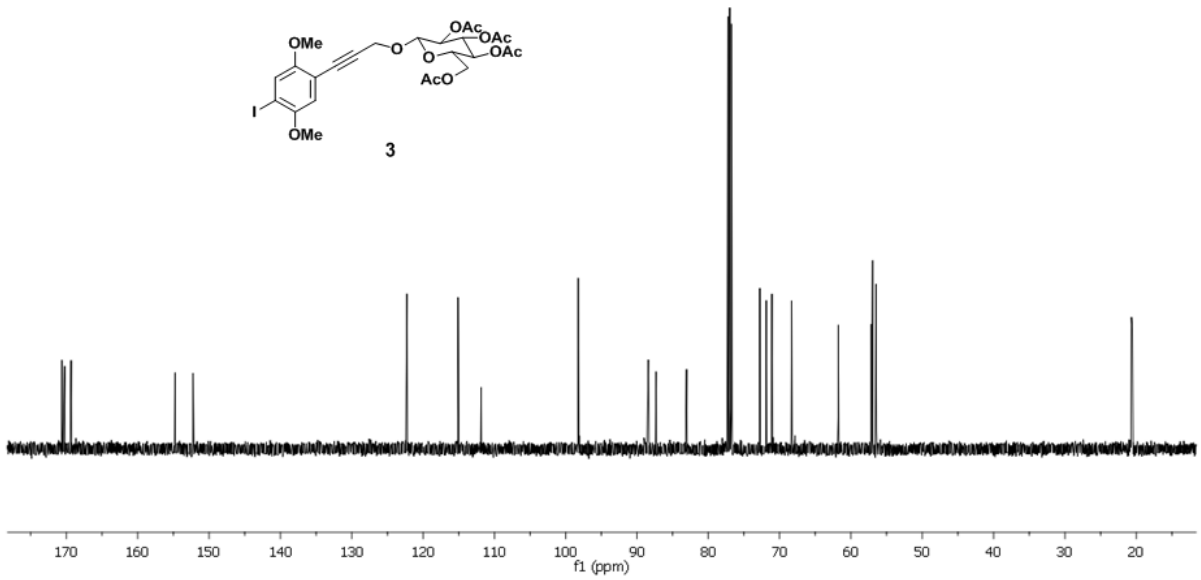
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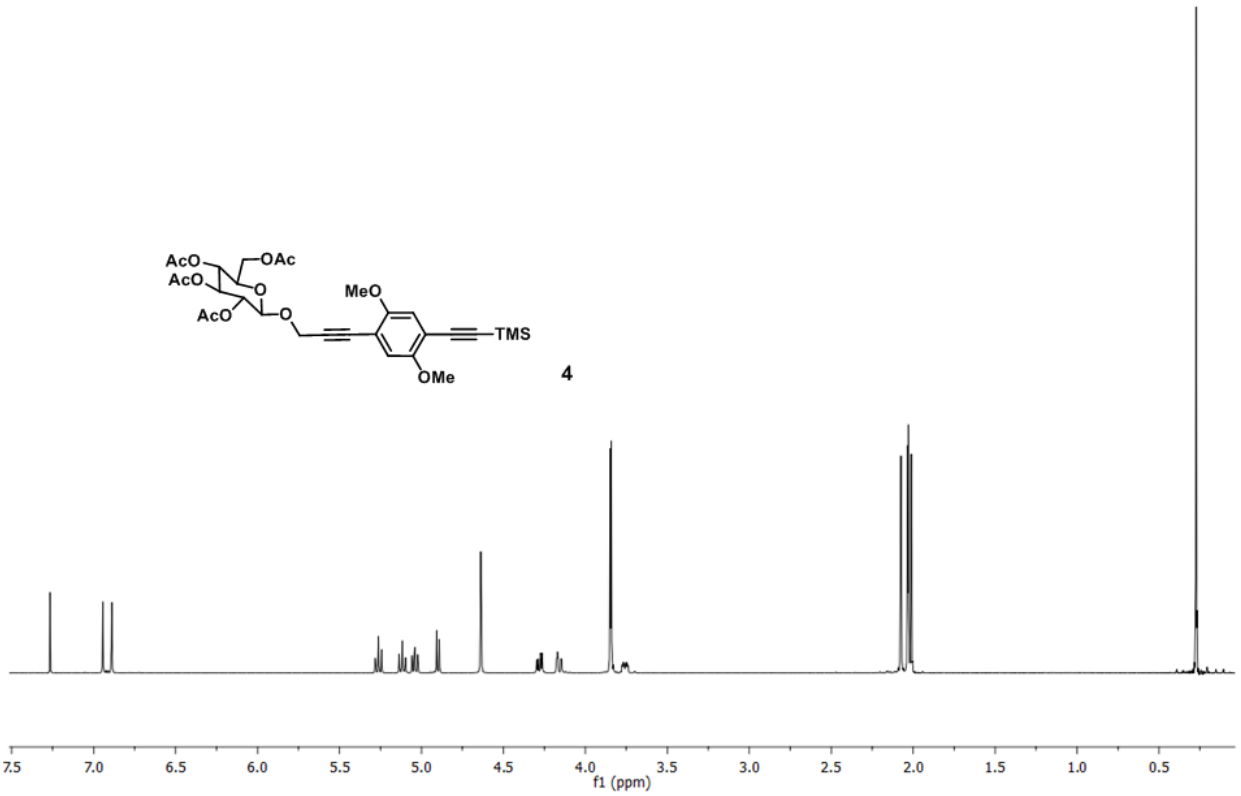
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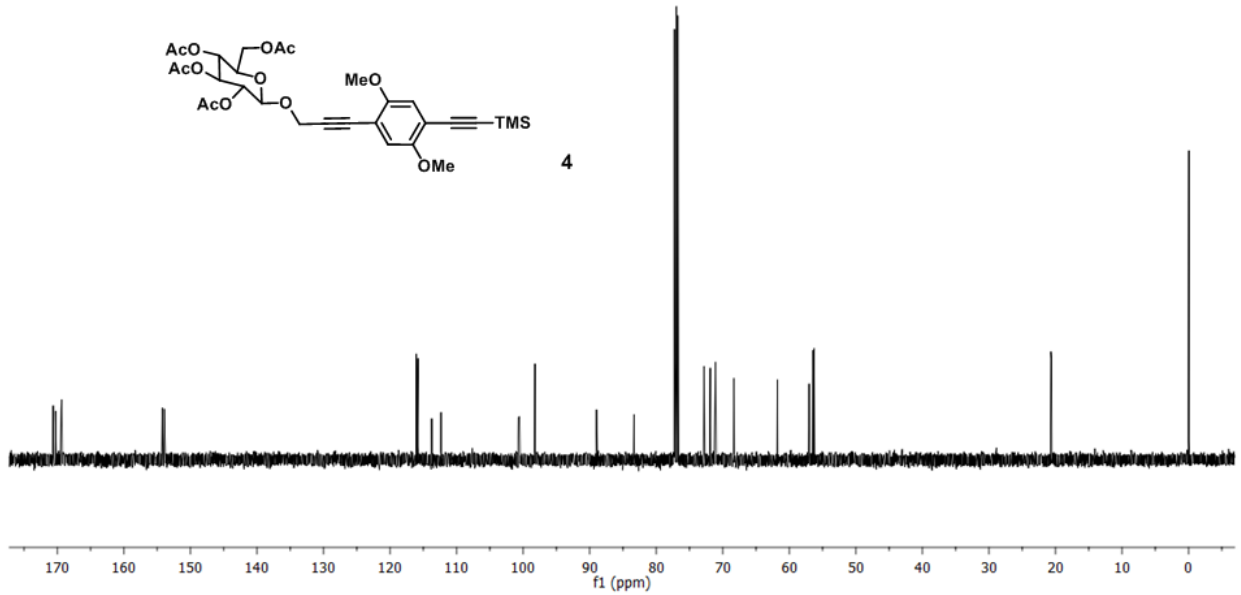
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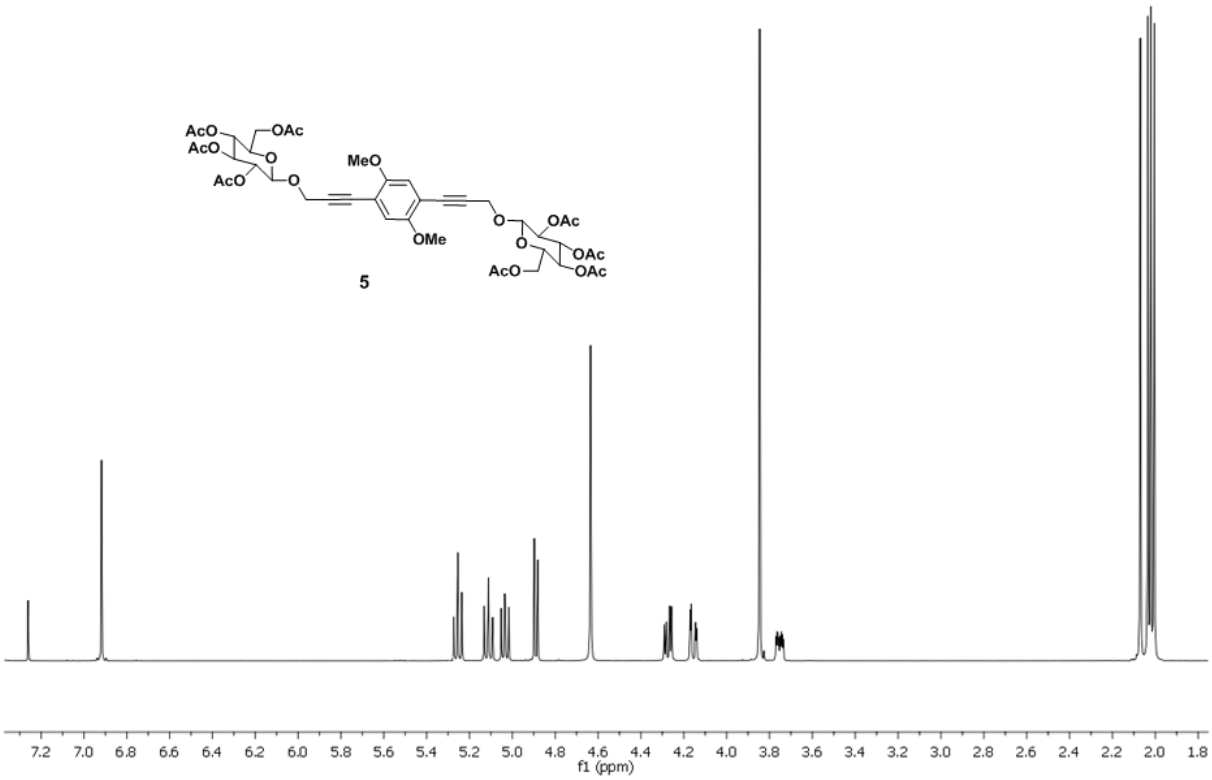
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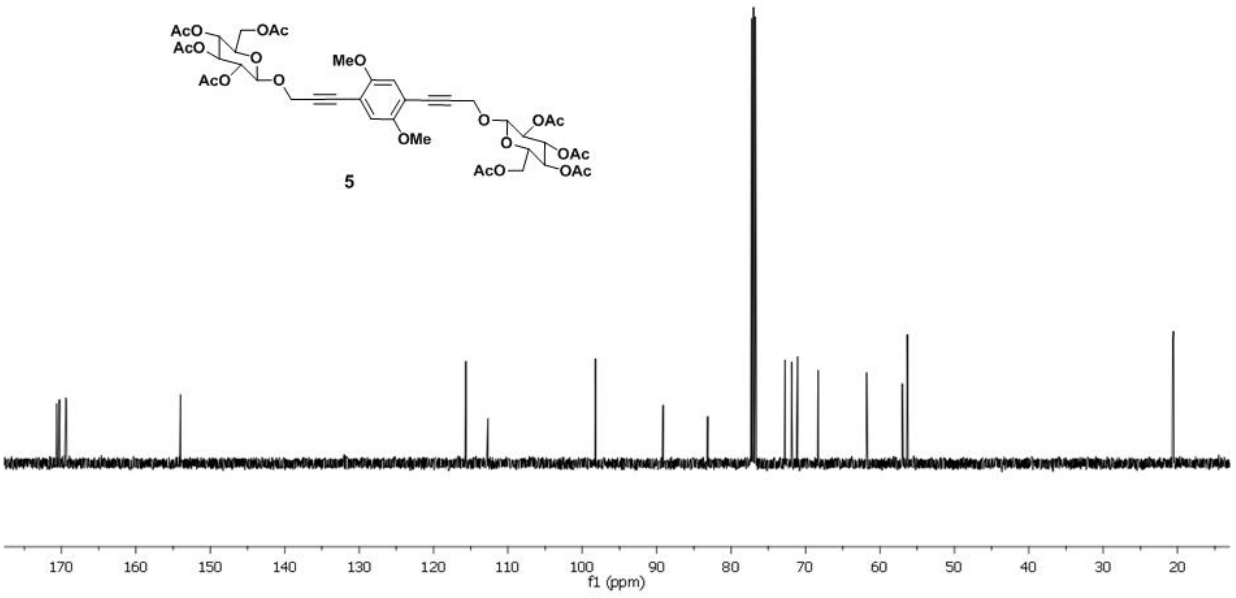
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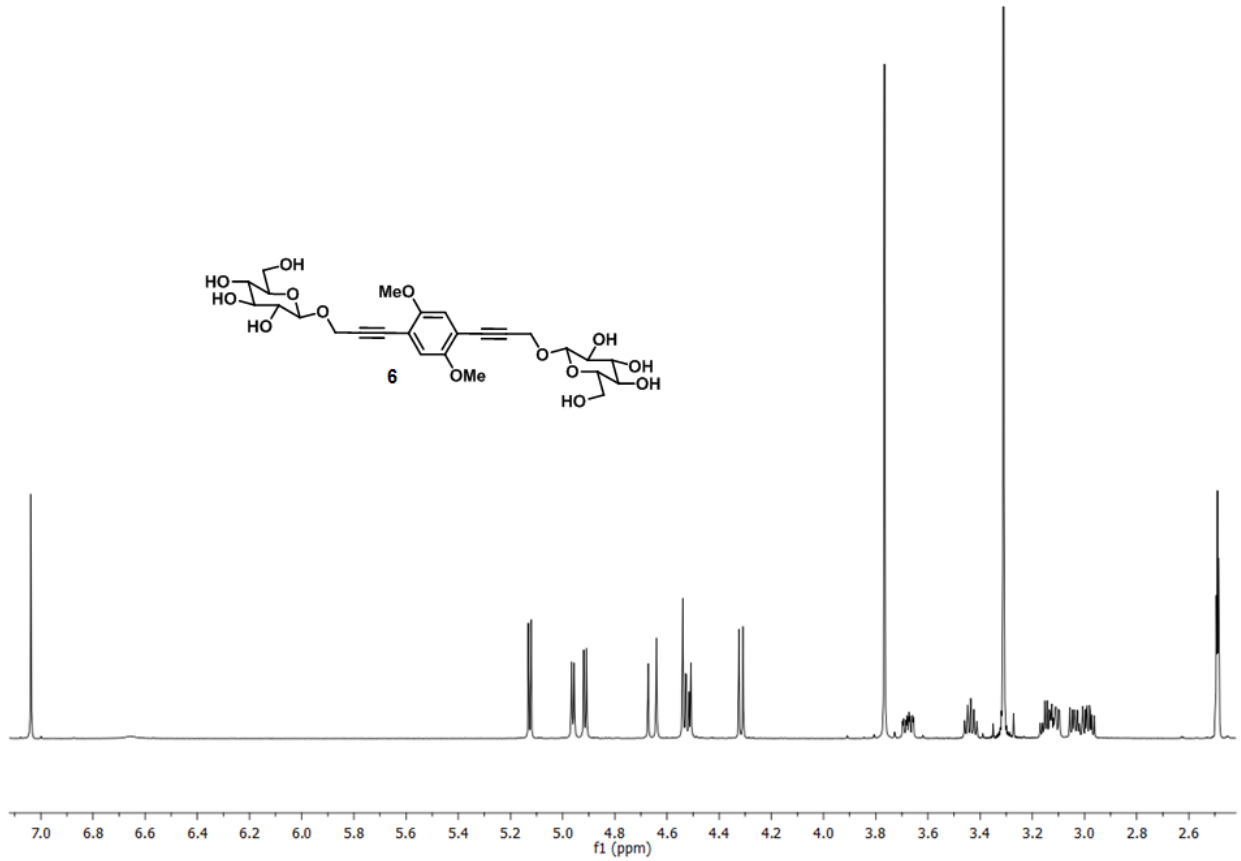
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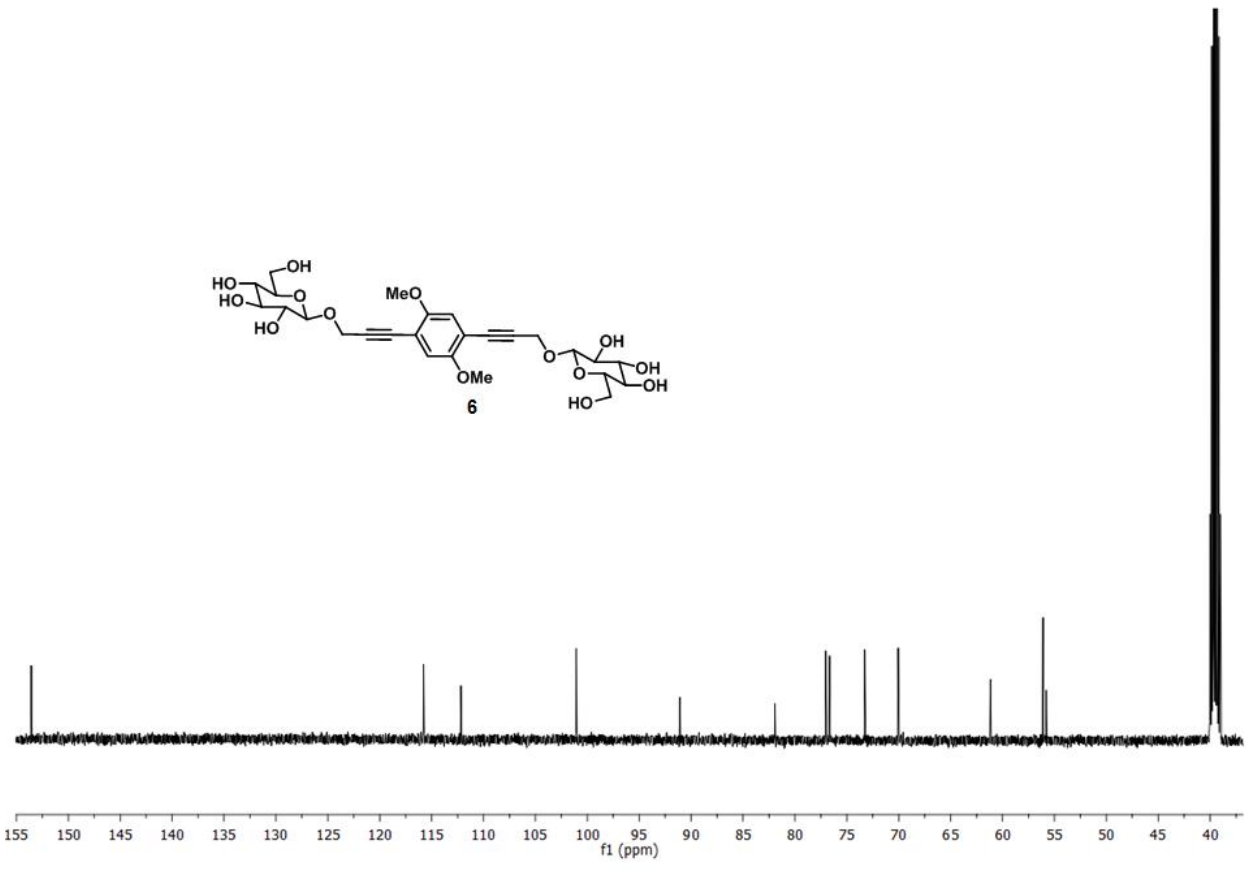
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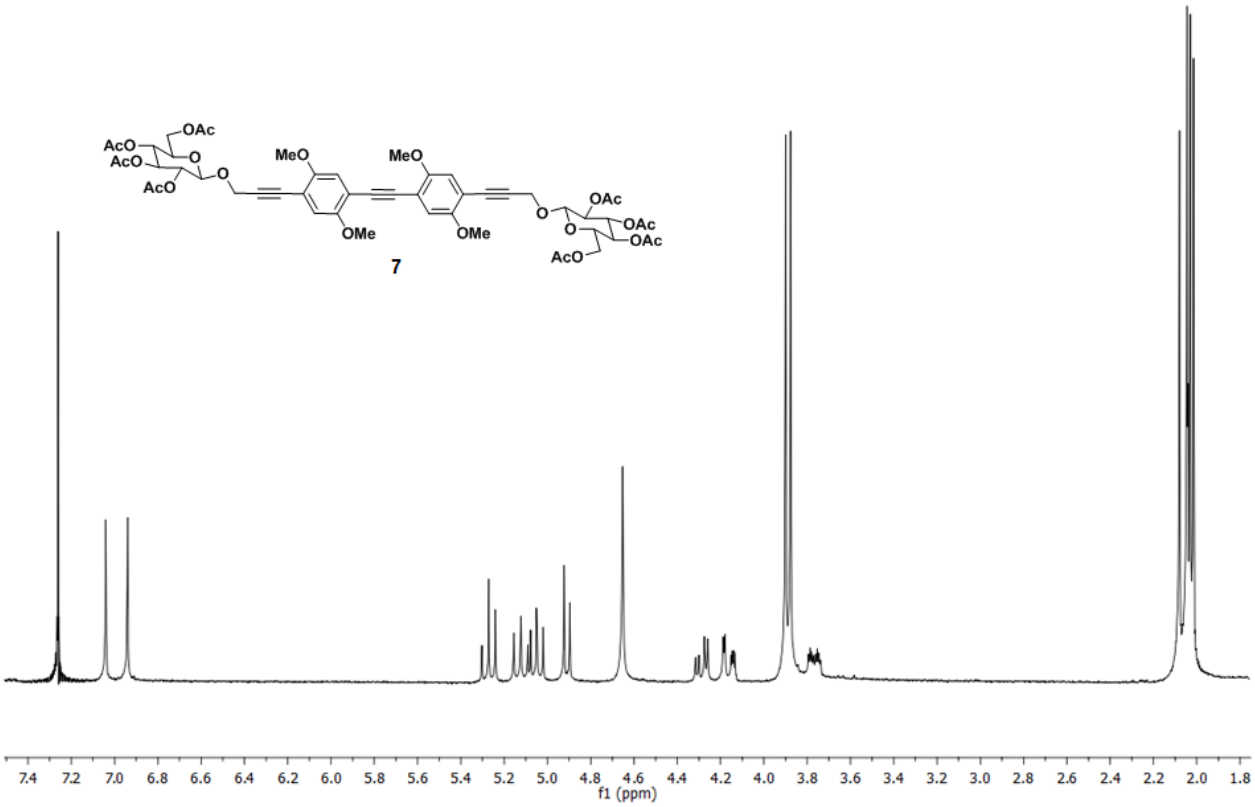
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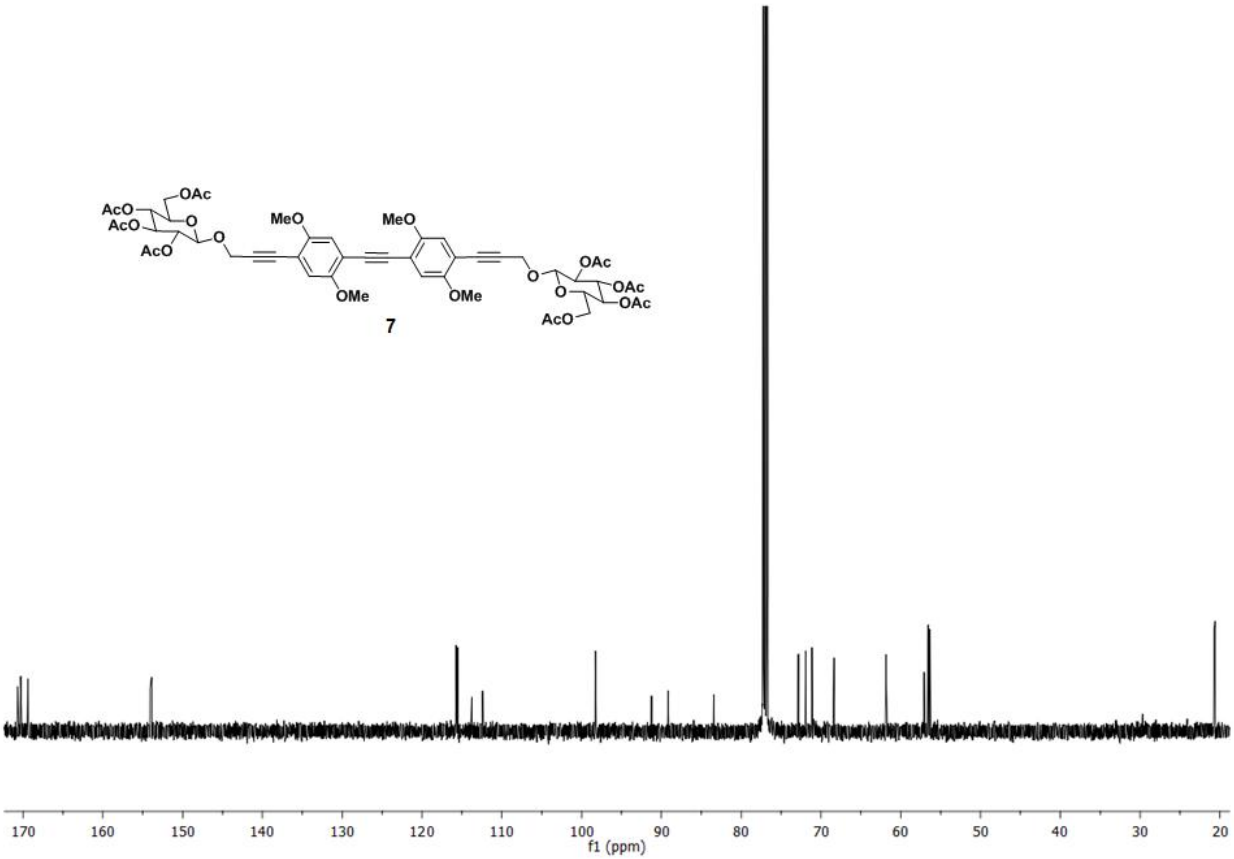
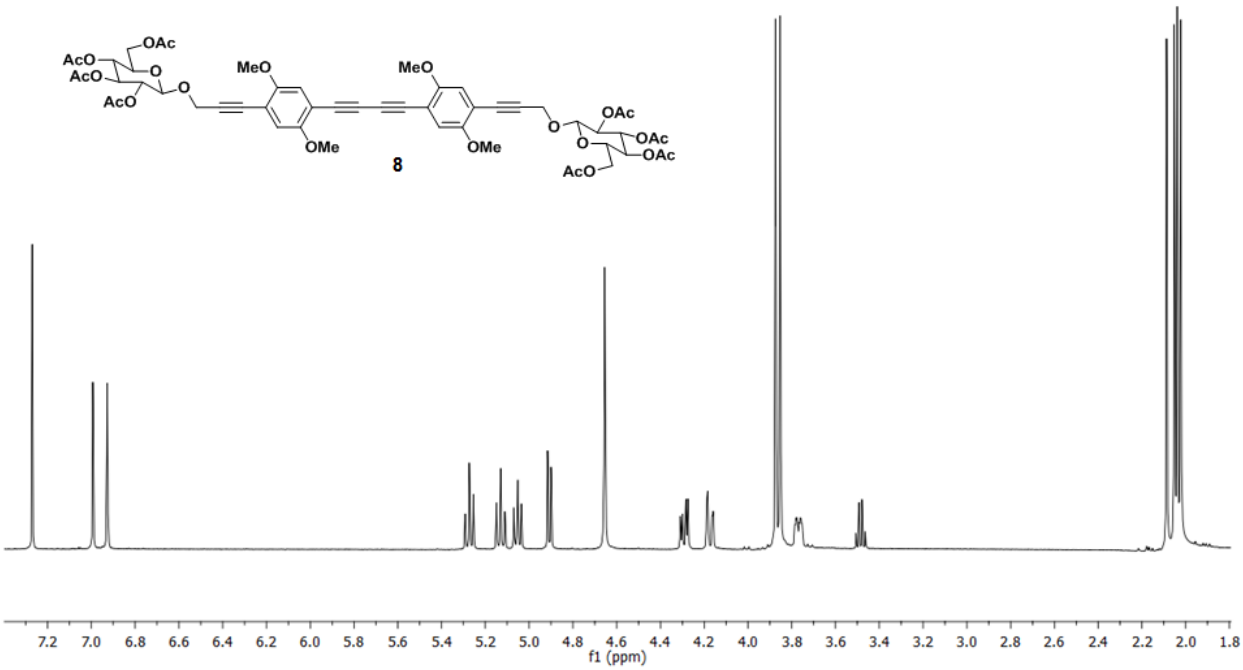
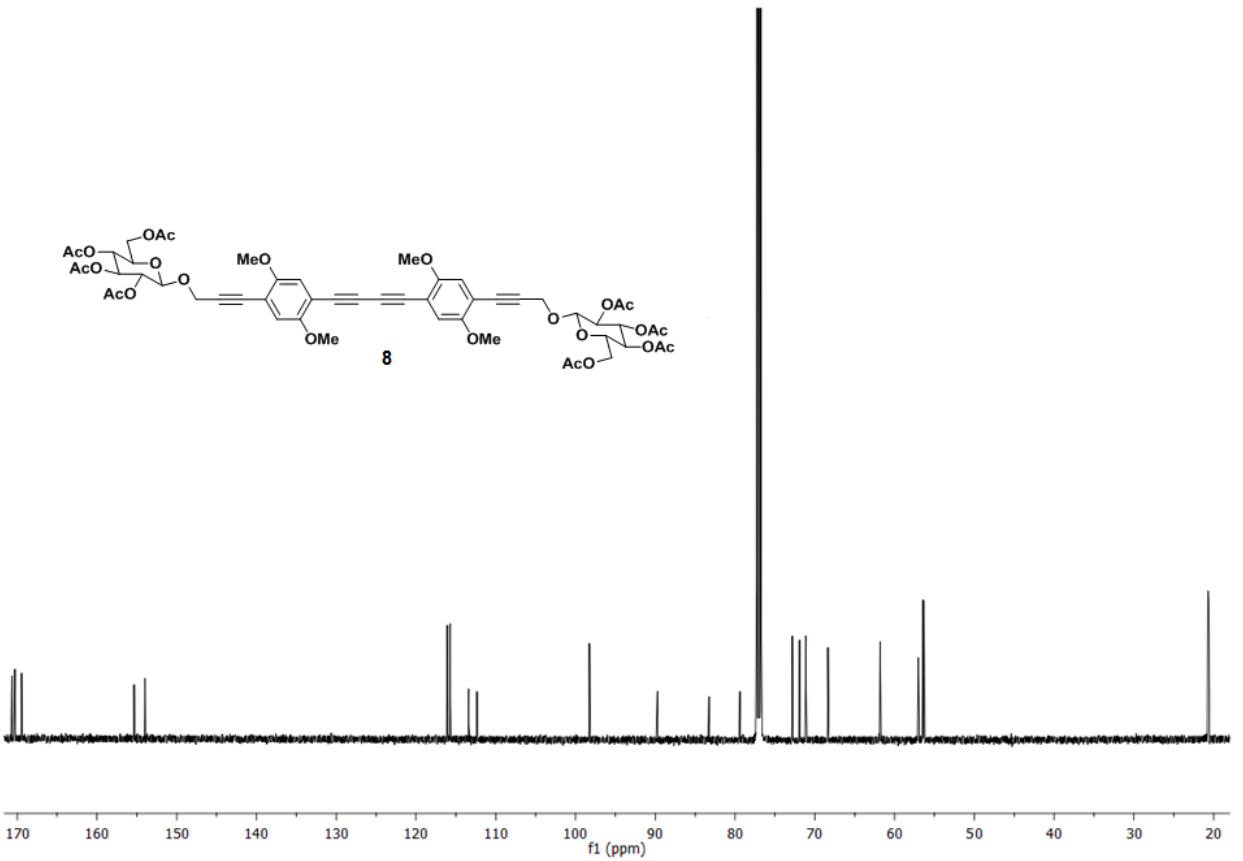
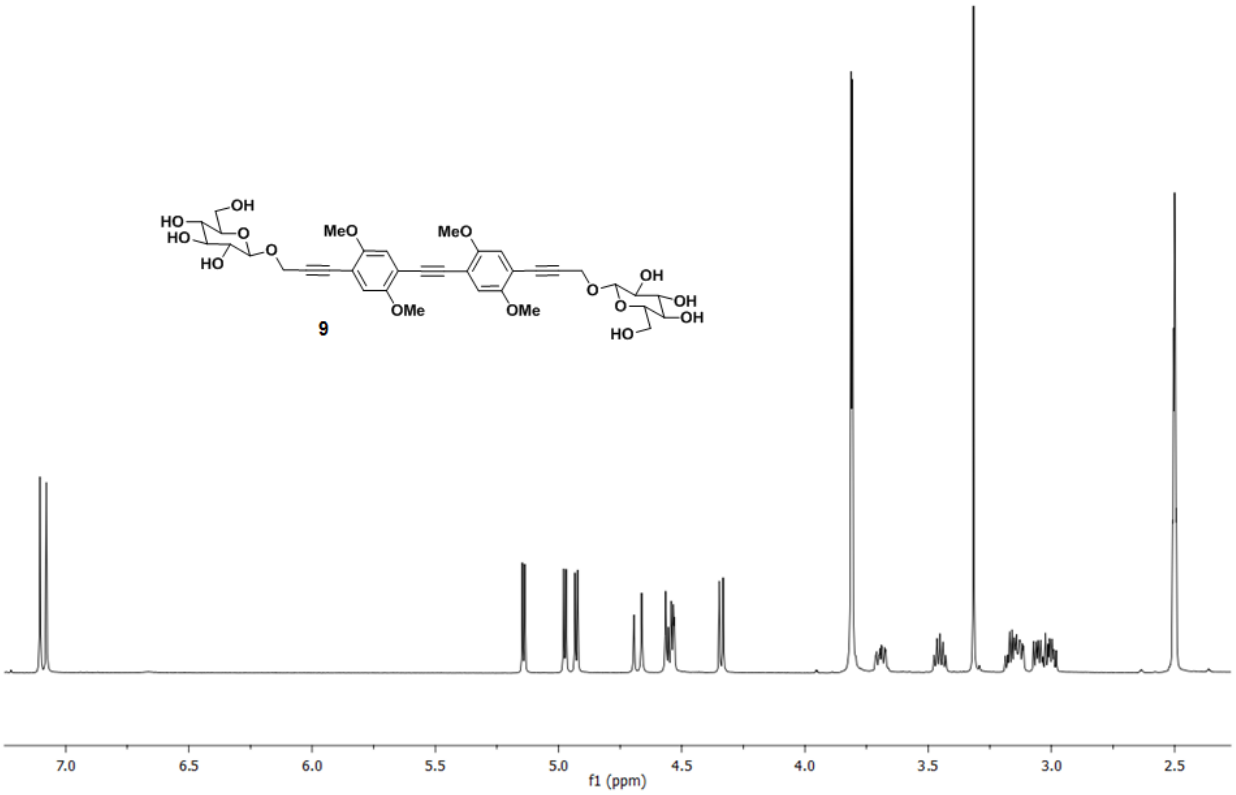
f1 (ppm)


OAc
AcO
AcO
AcO
O
O
MeO
MeO
OMe
OMe
OAc
OAc
OAc
O
AcO
7
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)


AcO
AcO
AcO
OAc
O
MeO
MeO
OMe
OMe
OAc
OAc
OAc
AcO
8
7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
f1 (ppm)


AcO
AcO
AcO
OAc
O
MeO
MeO
OMe
OMe
OAc
OAc
OAc
AcO
8
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)


OH
HO
HO
HO
O
O
MeO
MeO
OMe
OMe
OH
OH
OH
O
HO
9
7.0
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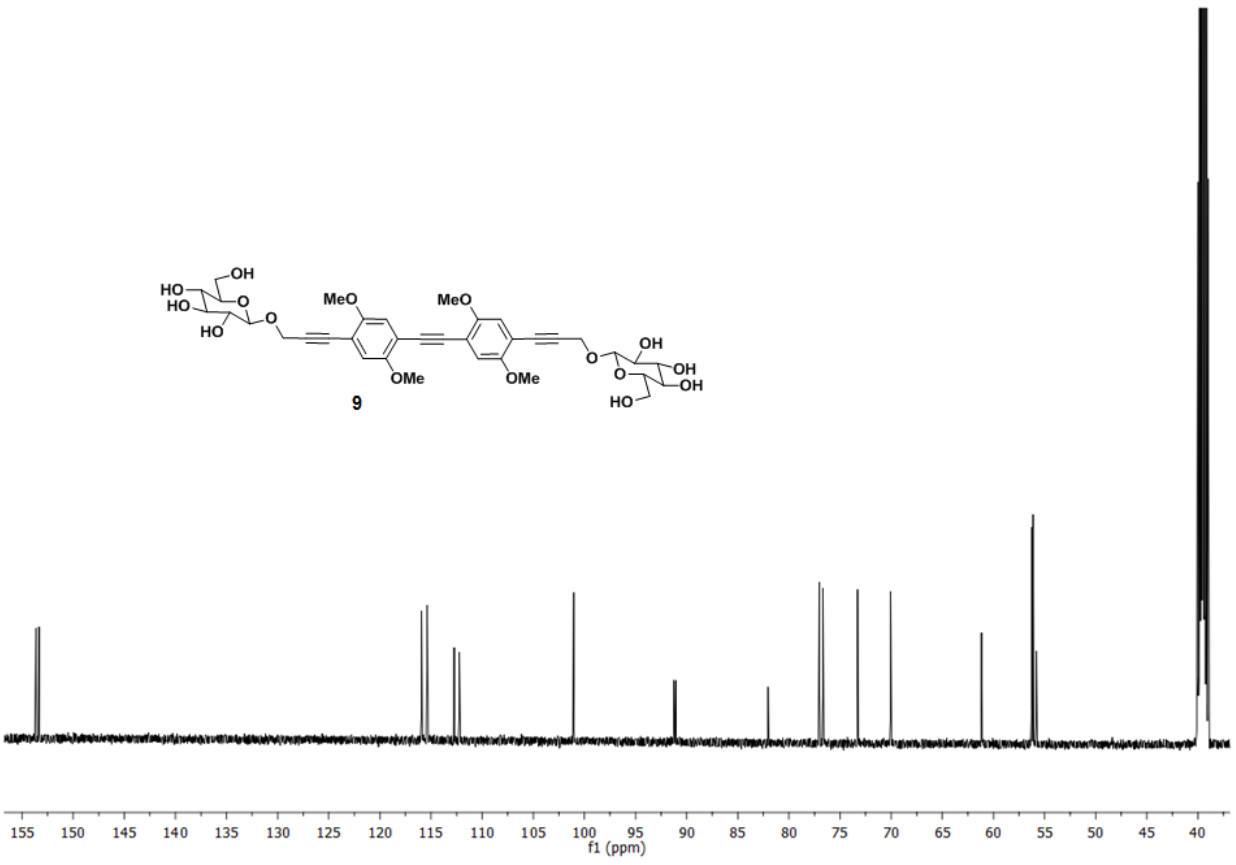
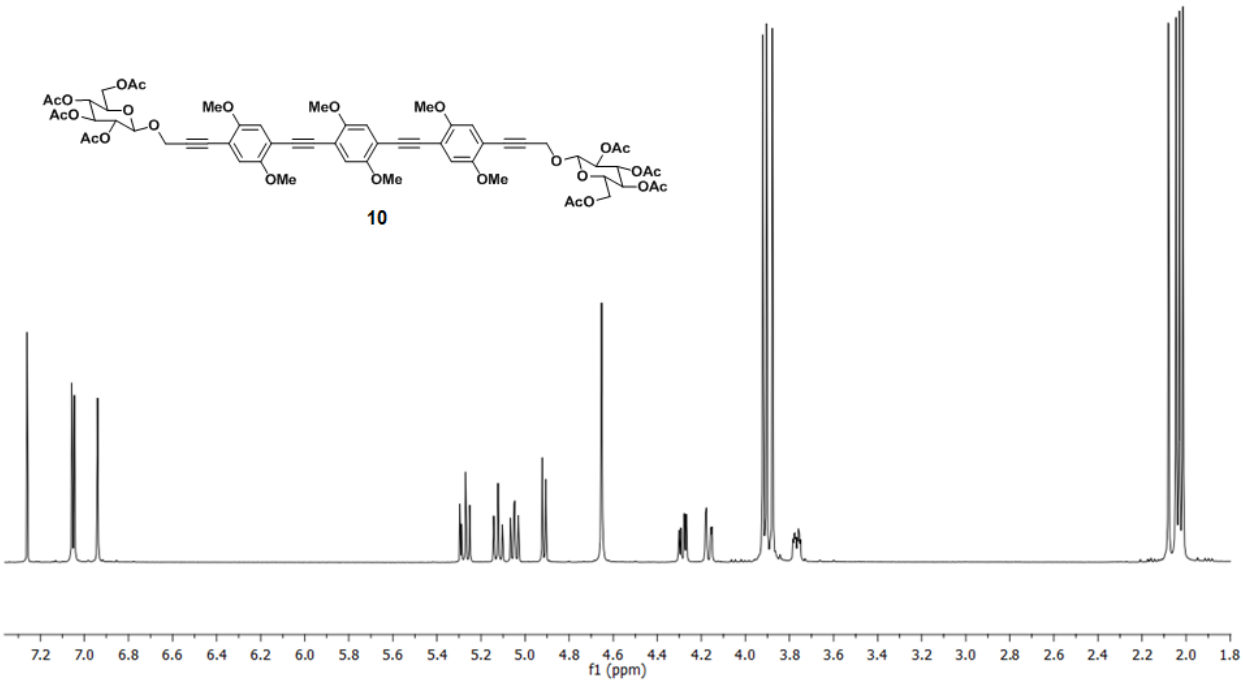
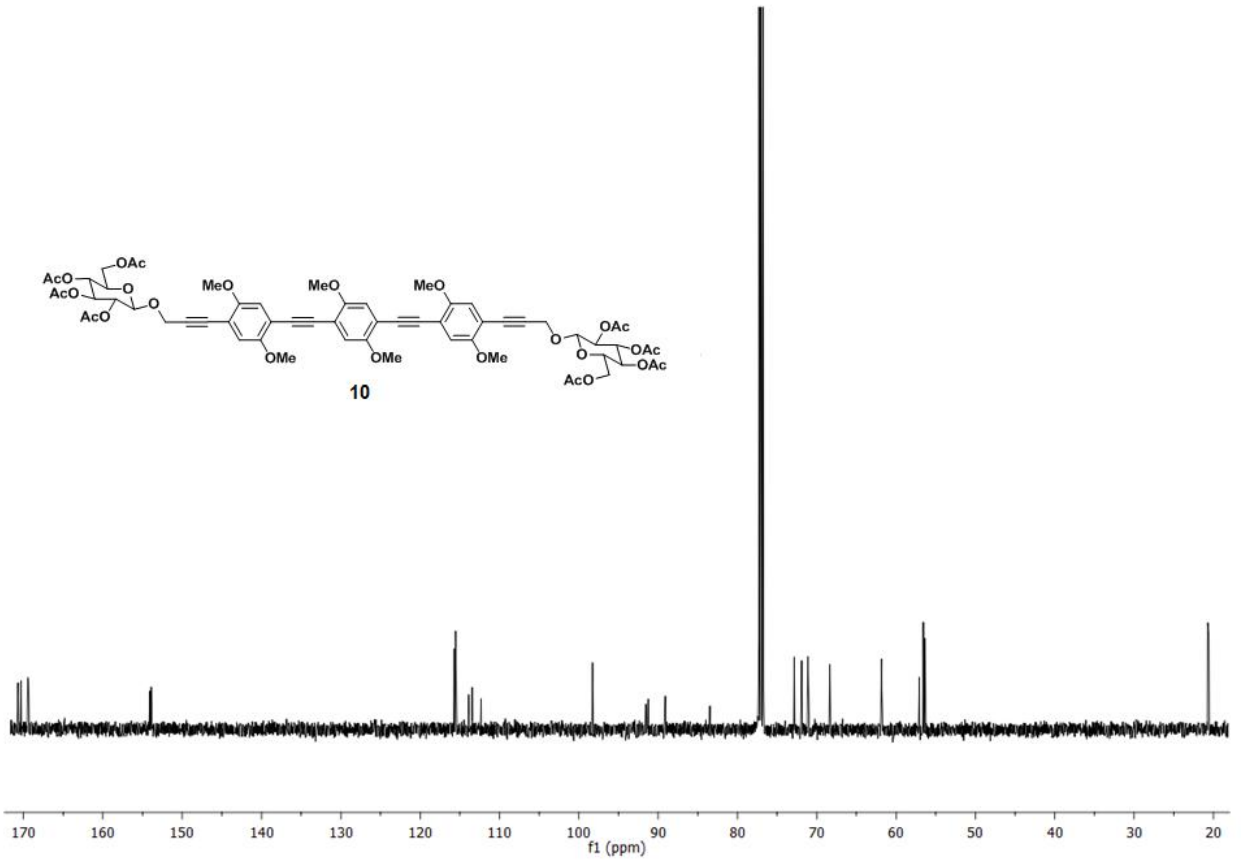
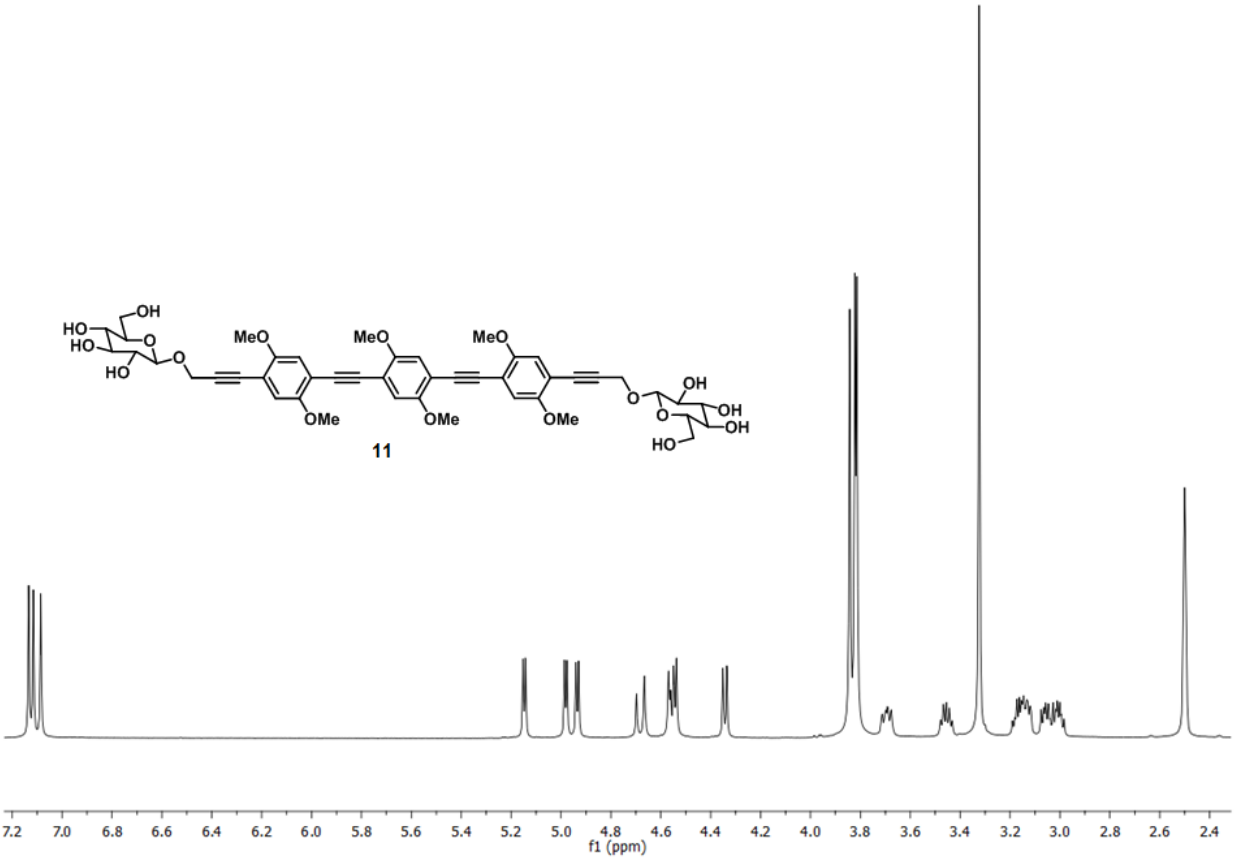
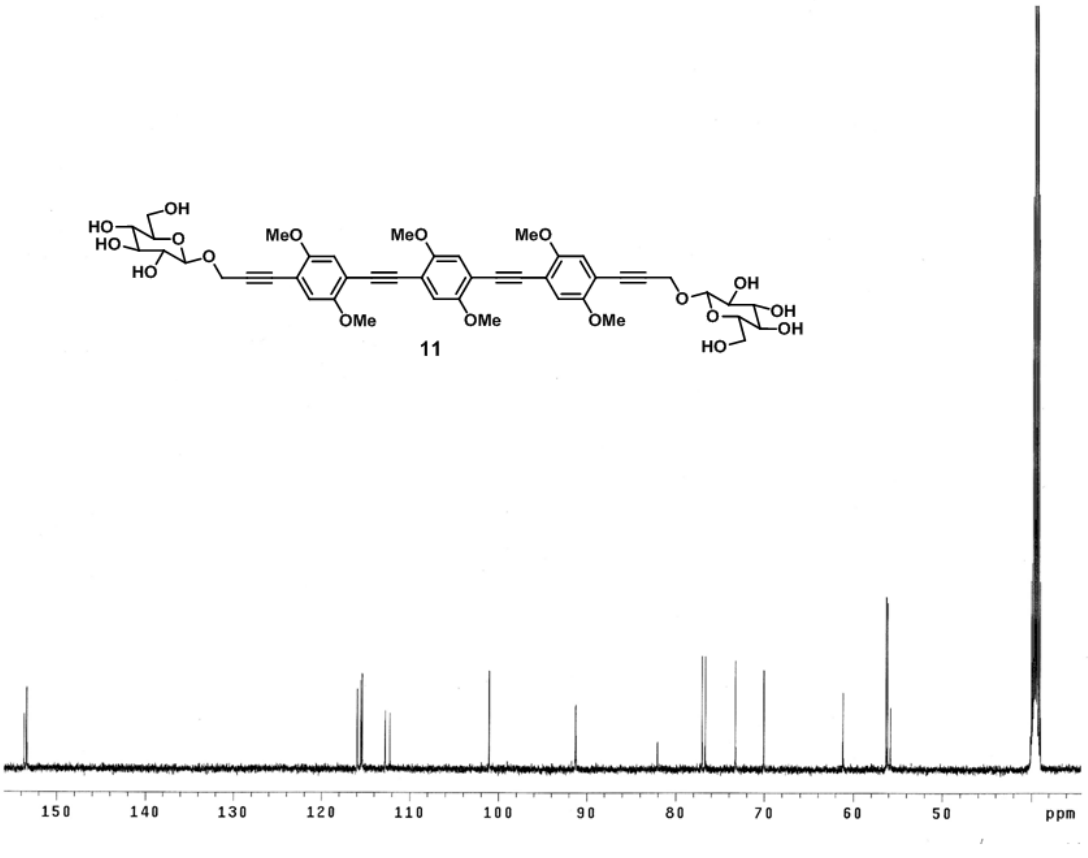
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f1 (ppm)

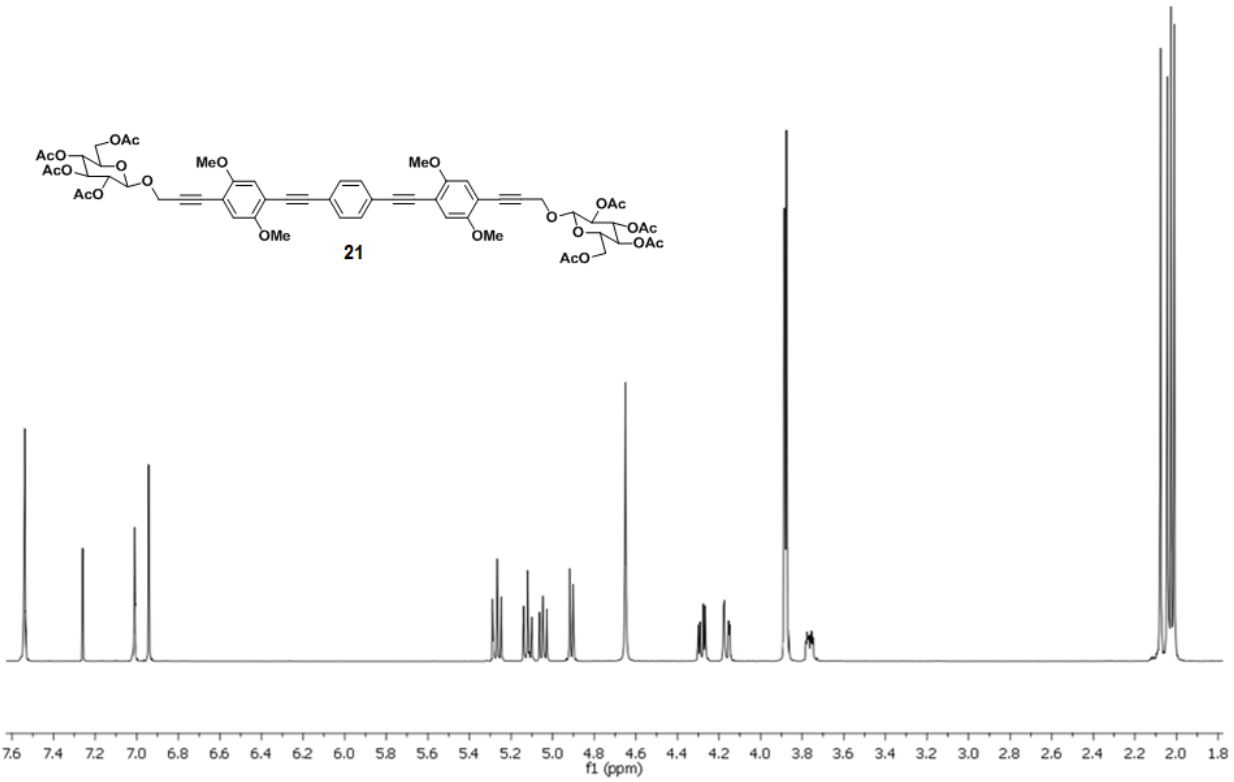

OH
HO
HO
HO
O
O
MeO
MeO
OMe
OMe
OH
OH
OH
O
HO
9
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
f1 (ppm)

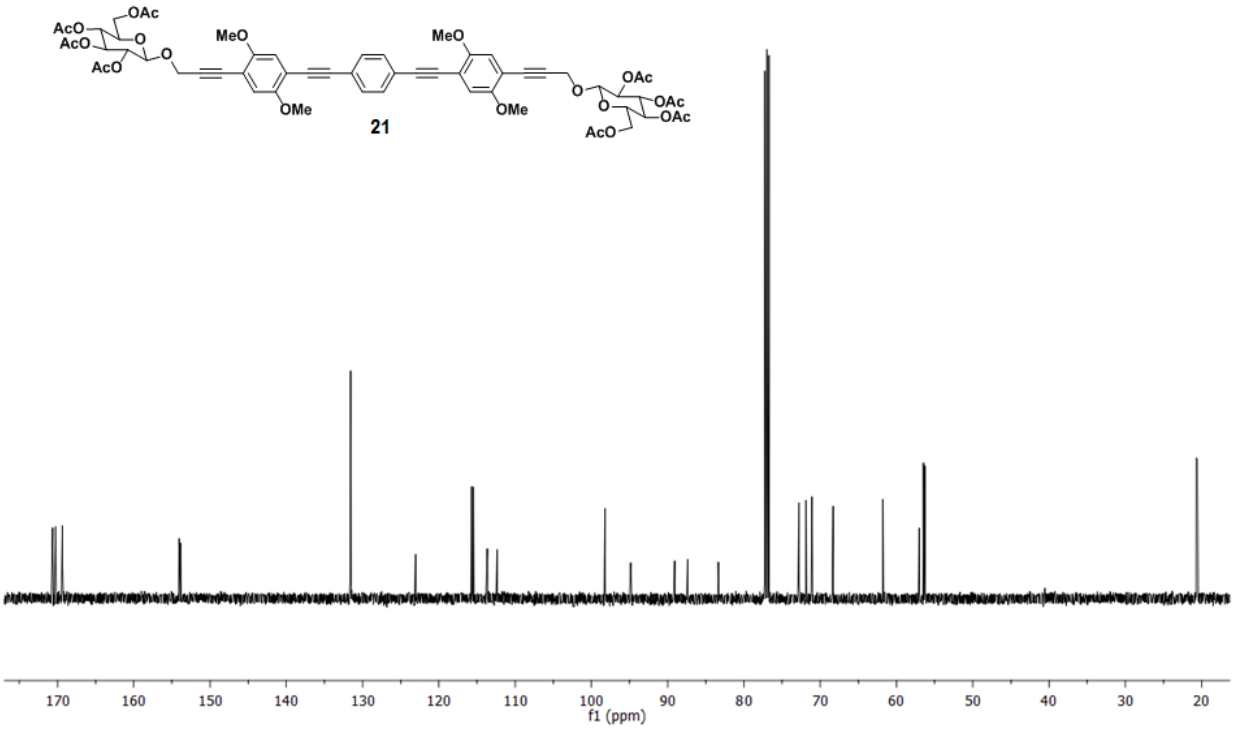

OAc
AcO
AcO
AcO
O
MeO
MeO
MeO
OMe
OMe
OMe
OAc
OAc
OAc
AcO
10
7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
f1 (ppm)

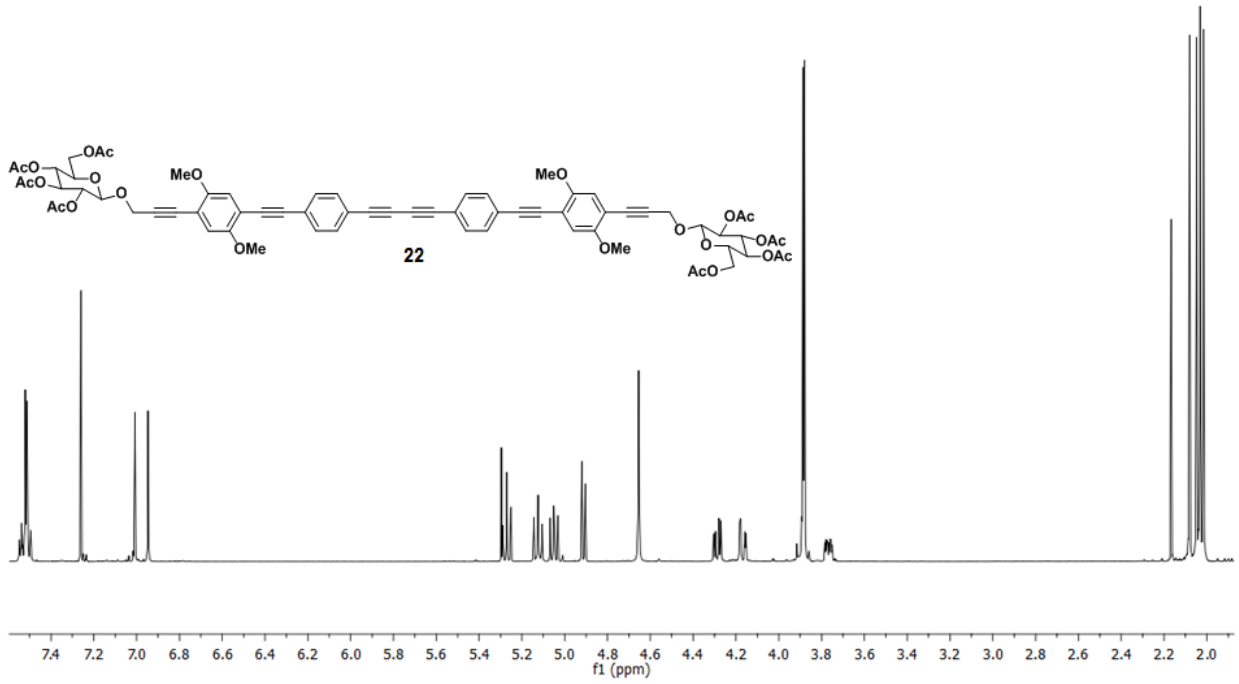

OAc
AcO
AcO
AcO
O
MeO
MeO
MeO
OMe
OMe
OMe
OAc
OAc
OAc
AcO
10
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

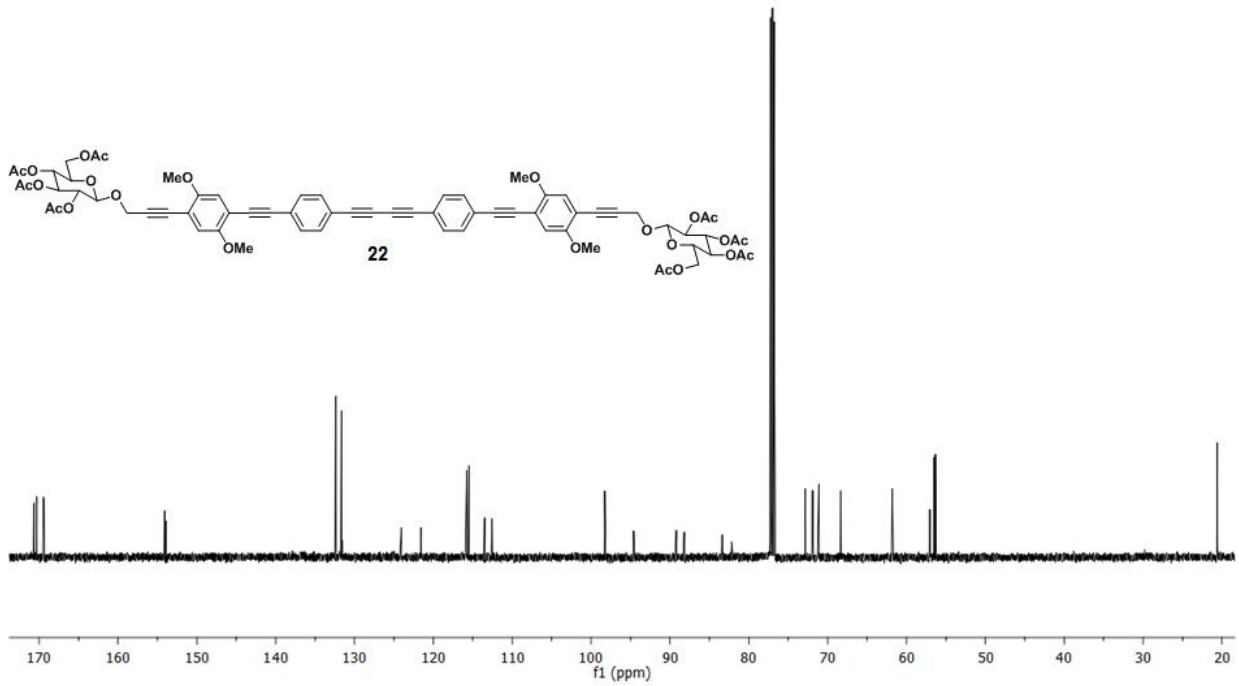

HO
HO
HO
OH
O
MeO
MeO
MeO
OMe
OMe
OMe
O
OH
OH
OH
O
HO
11
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
f1 (ppm)

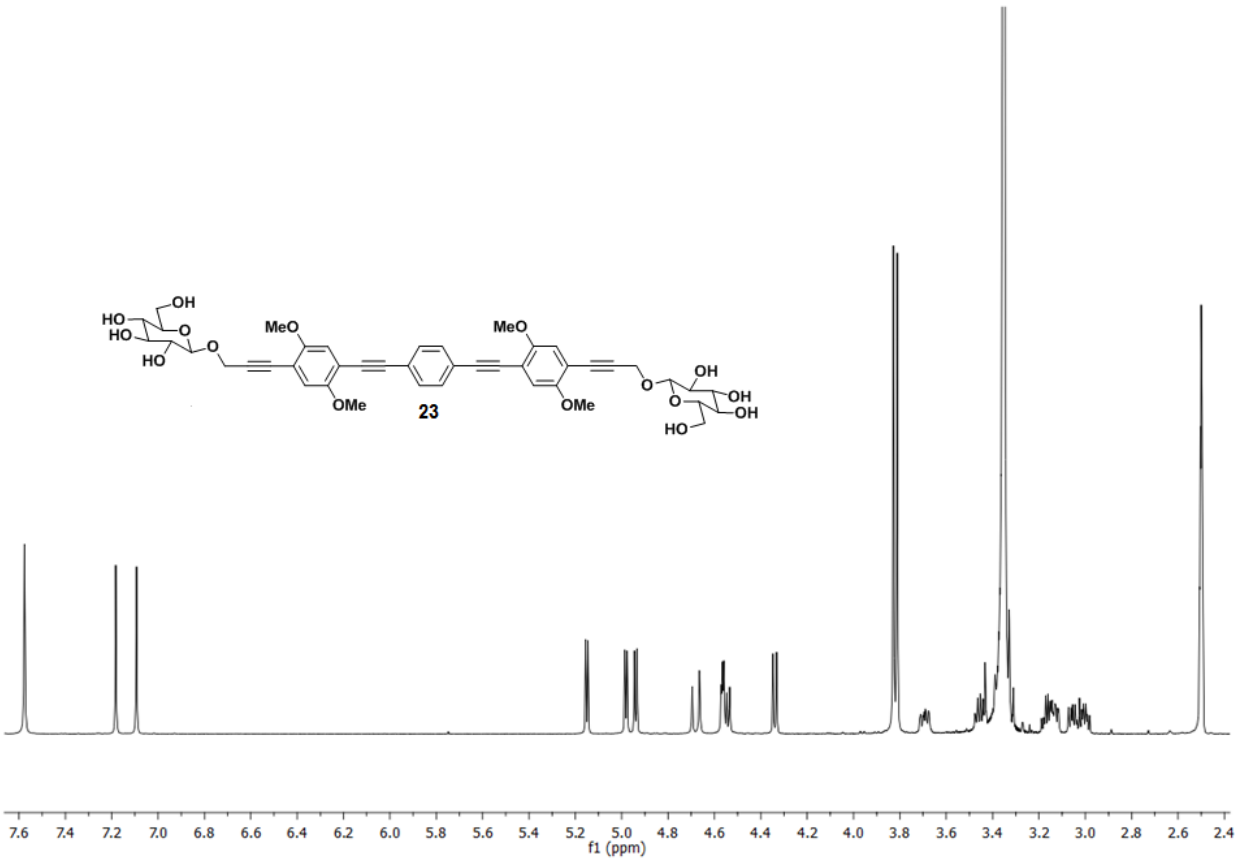

HO
HO
HO
OH
O
MeO
MeO
MeO
OMe
OMe
OMe
O
OH
OH
OH
O
HO
11
150
140
130
120
110
100
90
80
70
60
50
ppm

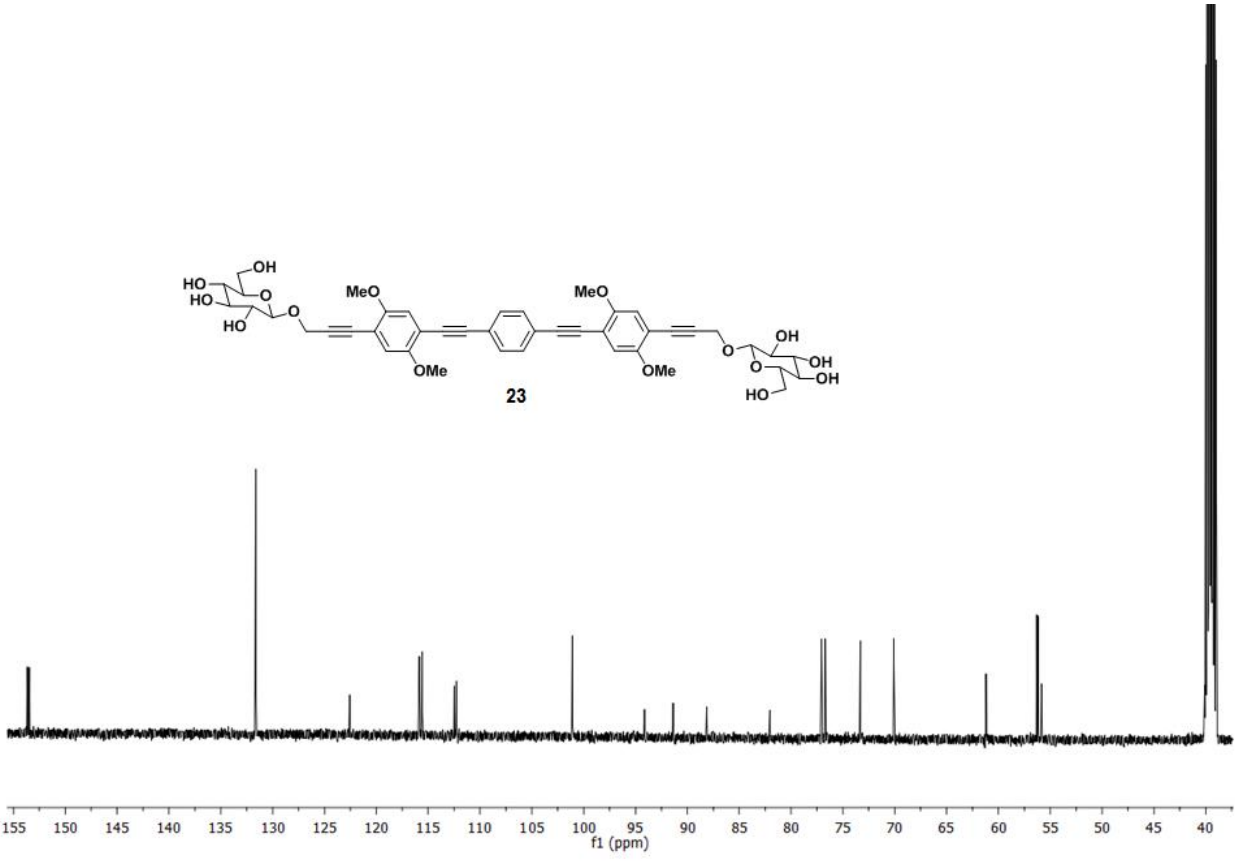

AcO
AcO
AcO
OAc
O
MeO
OMe
21
MeO
OMe
OAc
OAc
AcO
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
f1 (ppm)

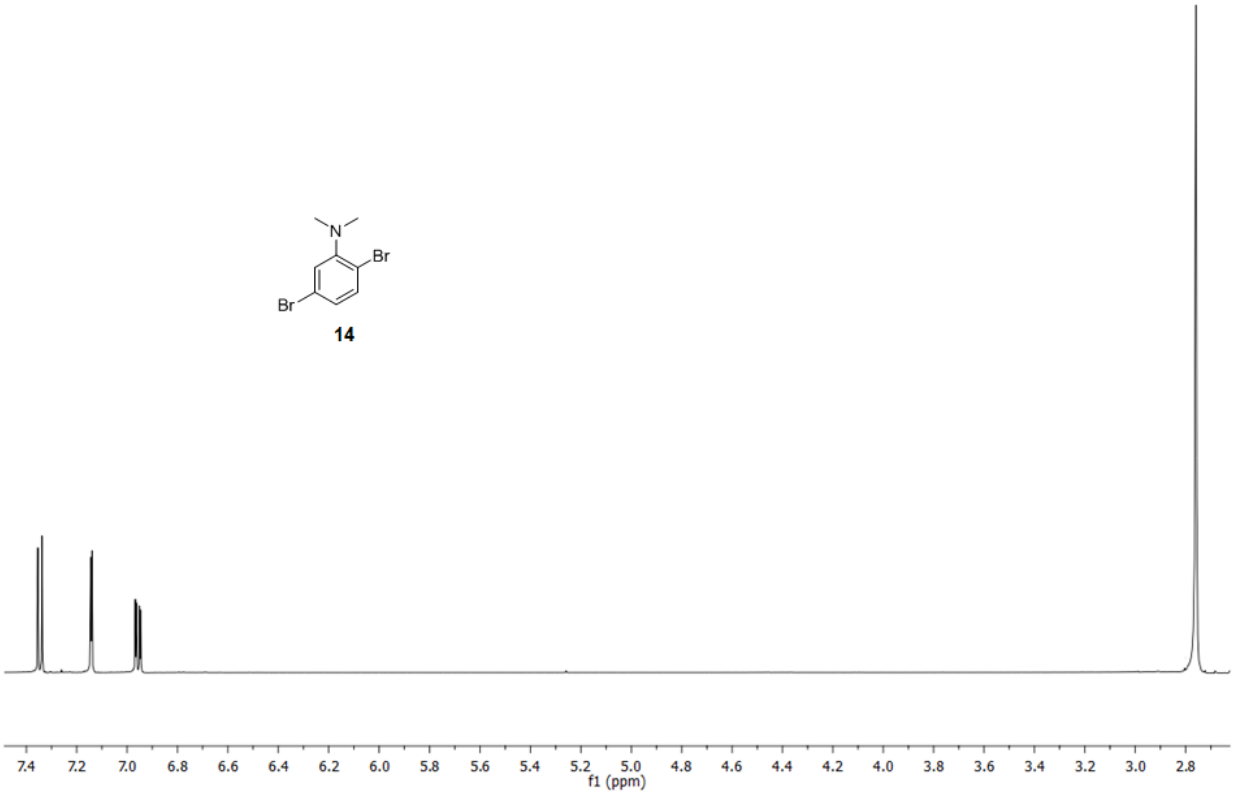

AcO
AcO
AcO
OAc
O
MeO
OMe
21
MeO
OMe
OAc
OAc
AcO
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

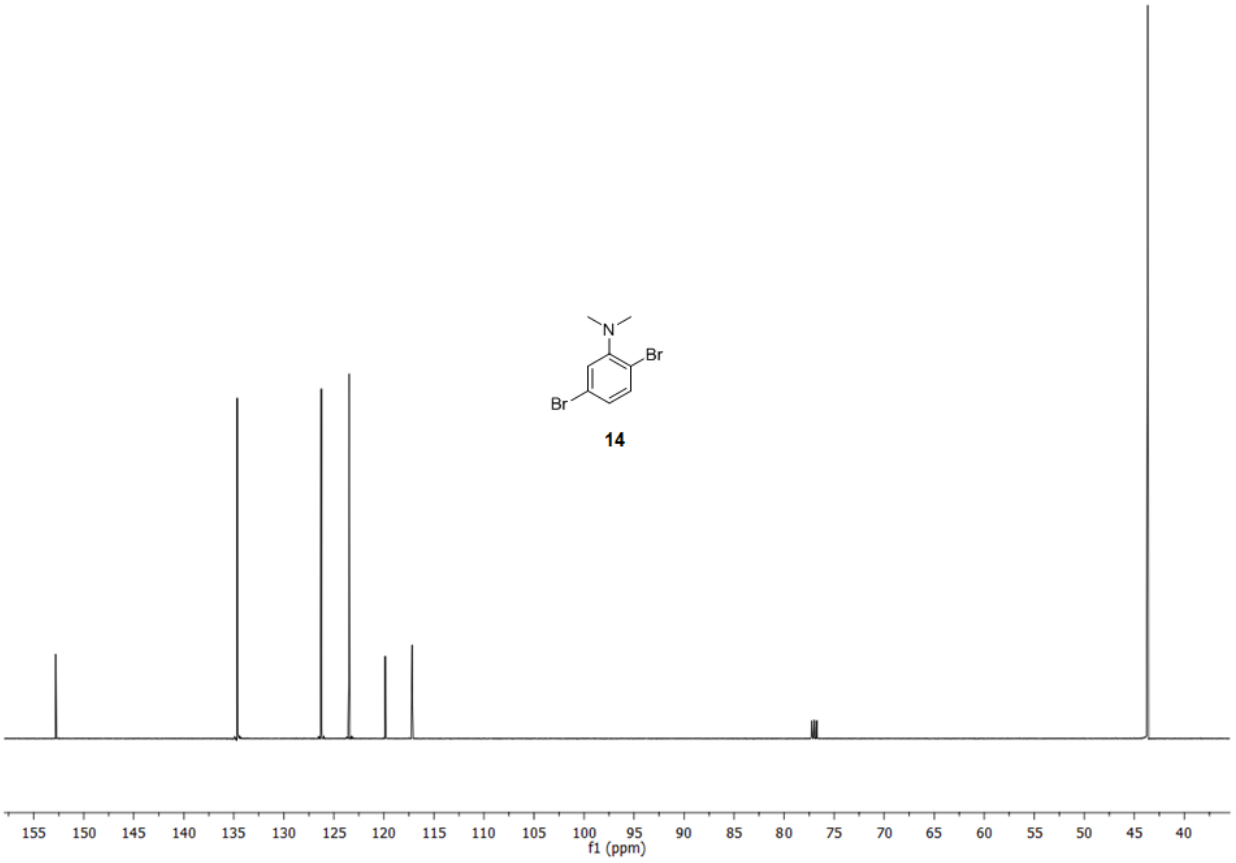

AcO
AcO
AcO
OAc
O
MeO
OMe
22
MeO
OMe
O
OAc
OAc
OAc
AcO
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0
f1 (ppm)

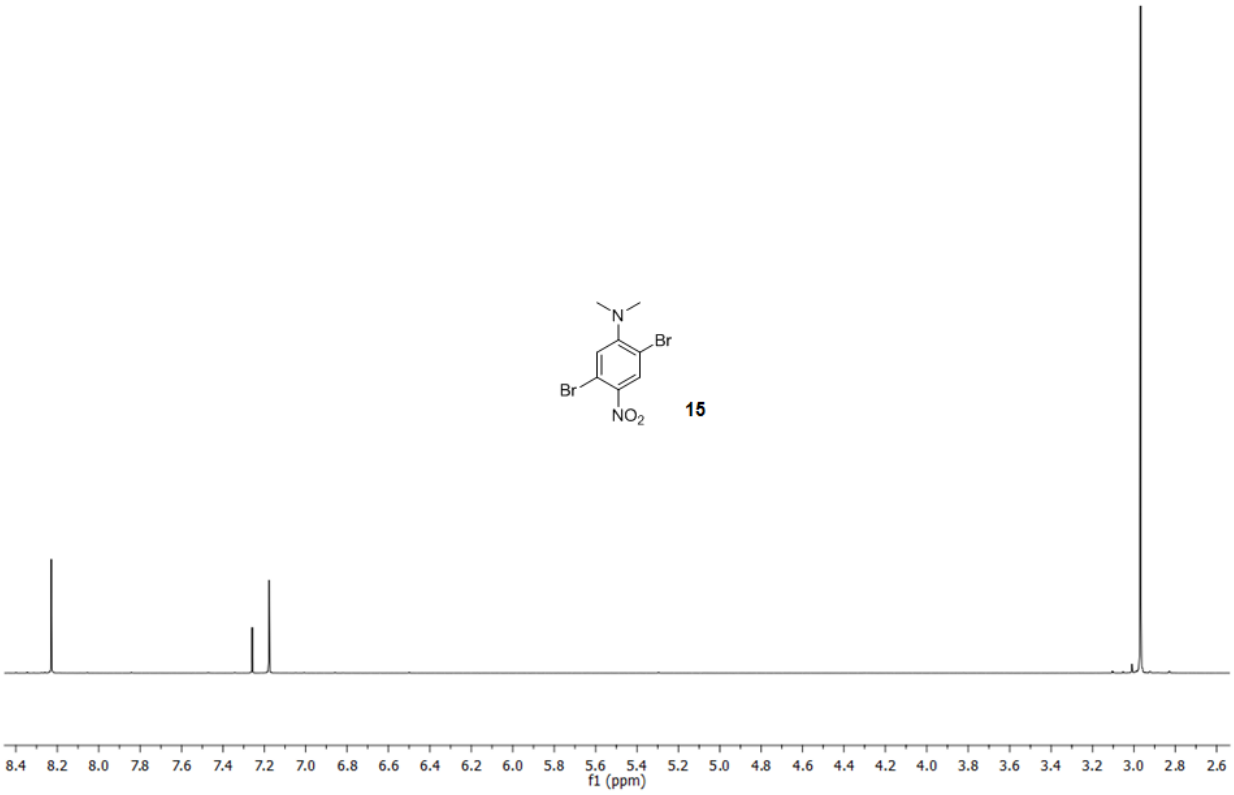

AcO
AcO
AcO
OAc
O
MeO
OMe
22
MeO
OMe
O
OAc
OAc
OAc
AcO
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

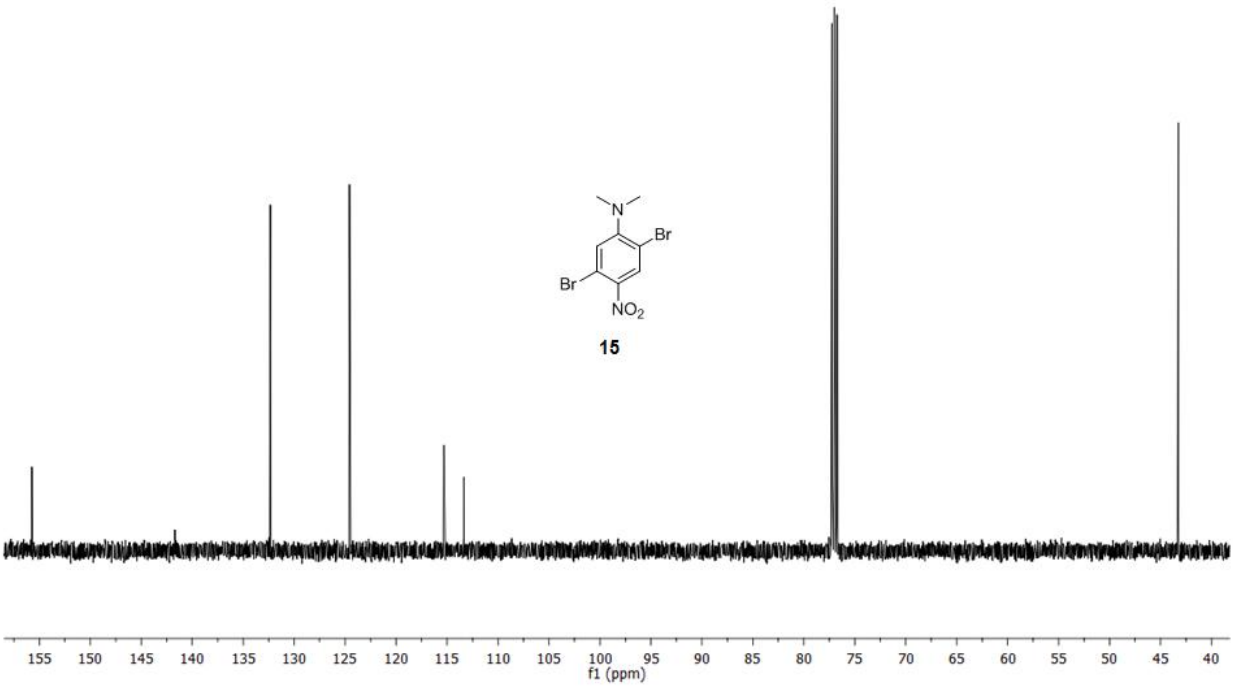

OH
HO
HO
HO
O
O
MeO
OMe
23
MeO
OMe
OH
OH
O
O
HO
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4
f1 (ppm)

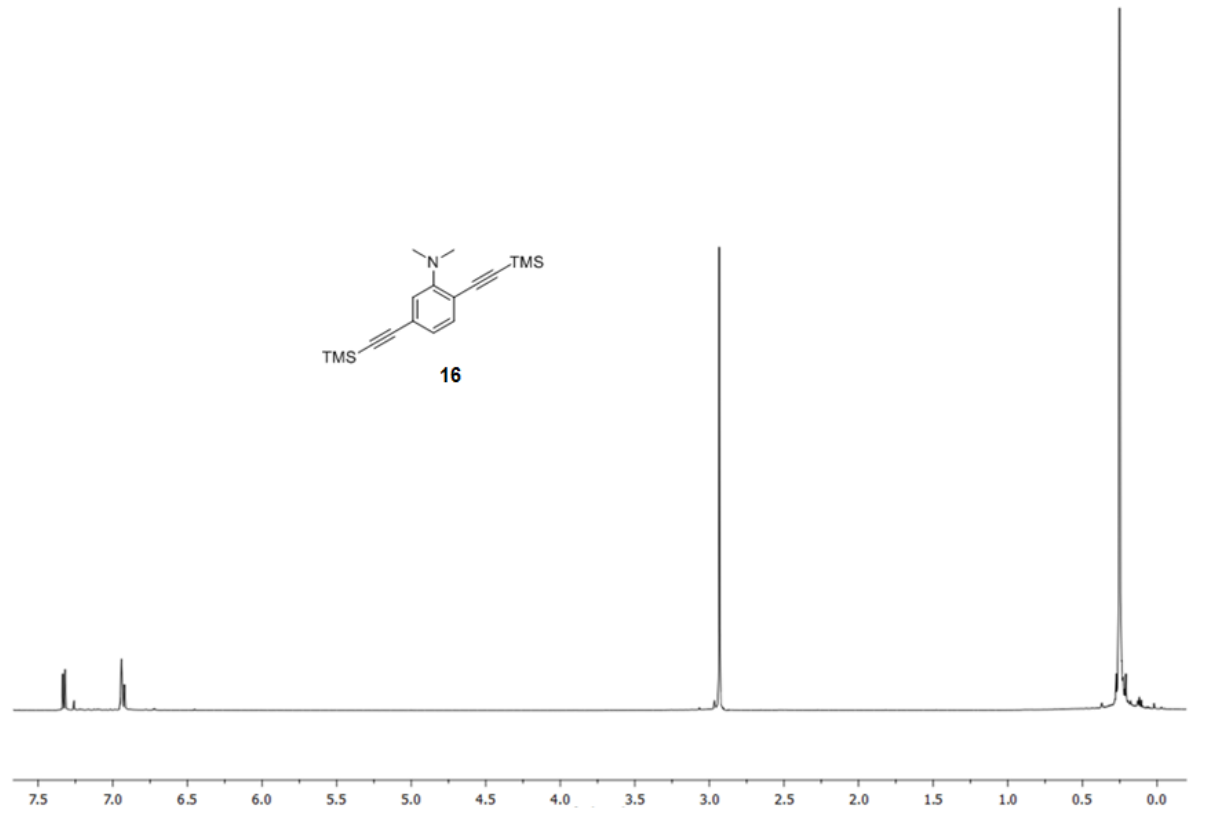

OH
HO
HO
HO
O
O
MeO
OMe
23
MeO
OMe
OH
OH
O
O
HO
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40
f1 (ppm)

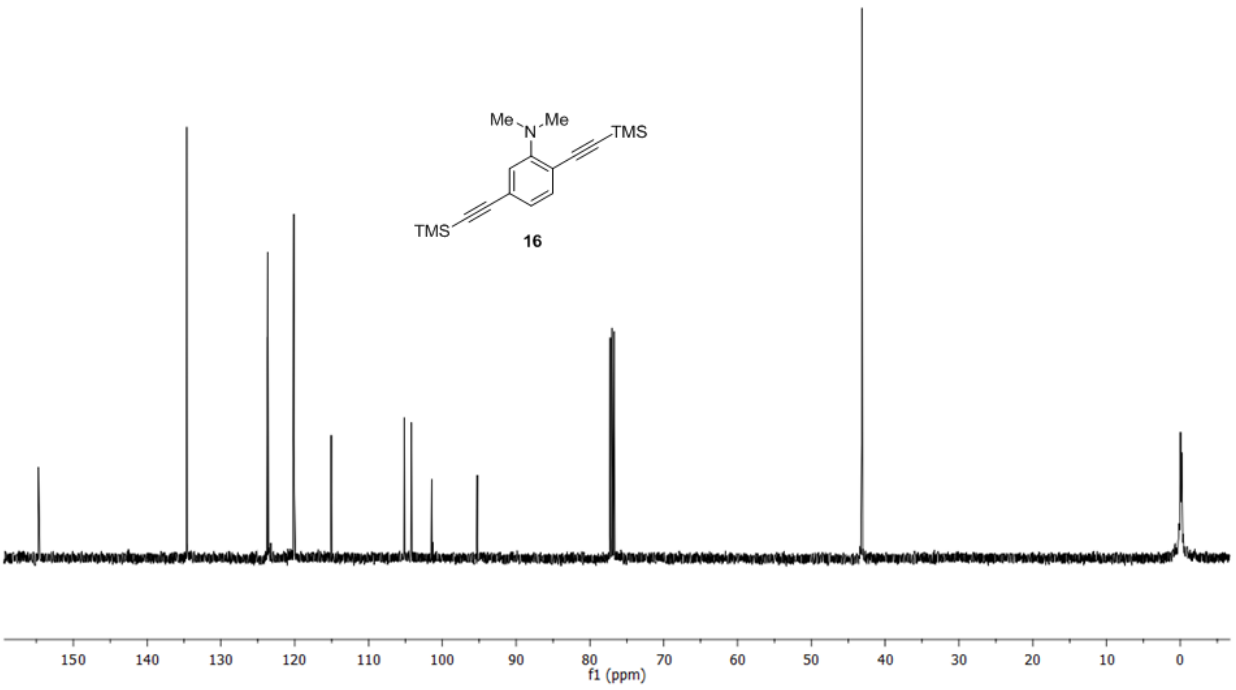

N
Br
Br
14
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8
f1 (ppm)

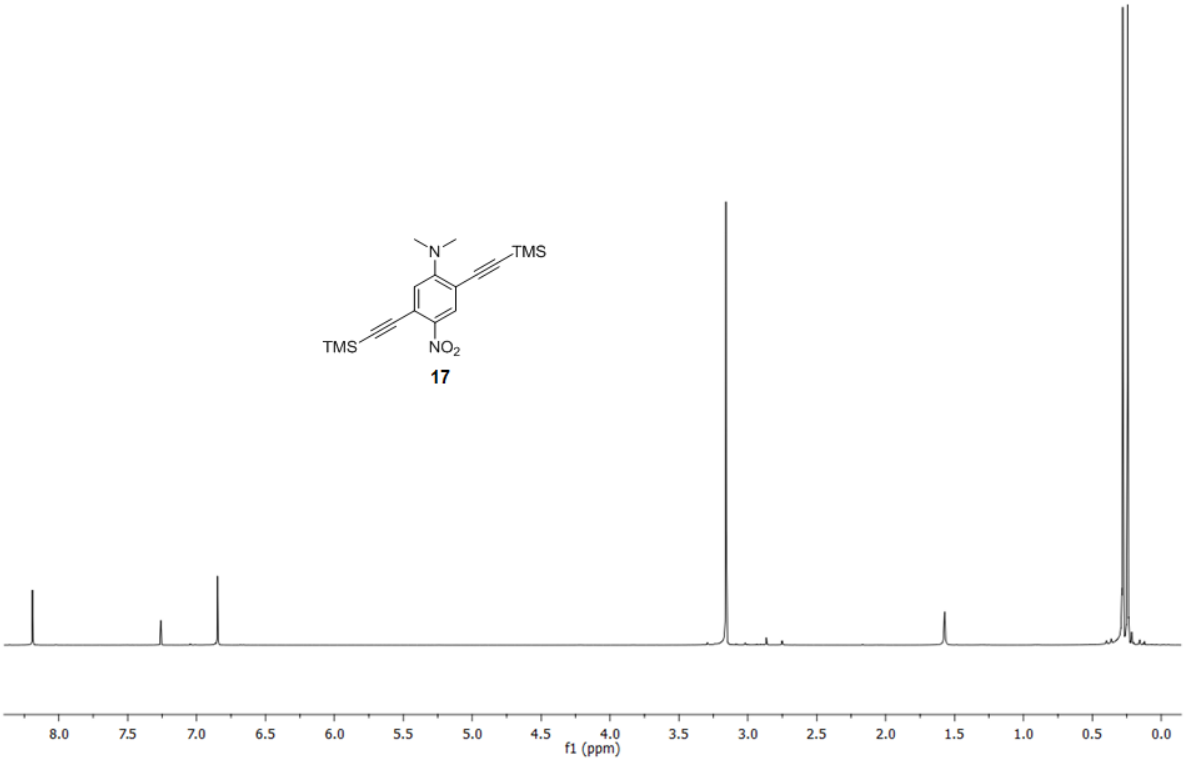

N
Br
Br
14
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40
f1 (ppm)


N
Br
Br
NO2
15
8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6
f1 (ppm)

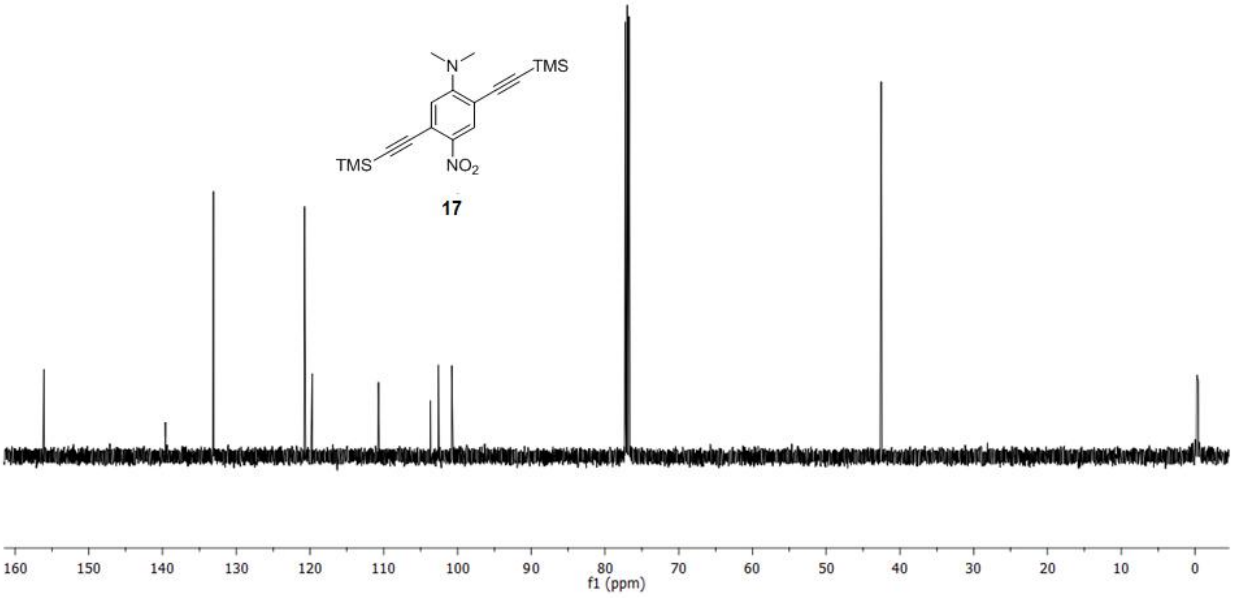

N
Br
Br
NO2
15
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40
f1 (ppm)


N
TMS
TMS
16
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

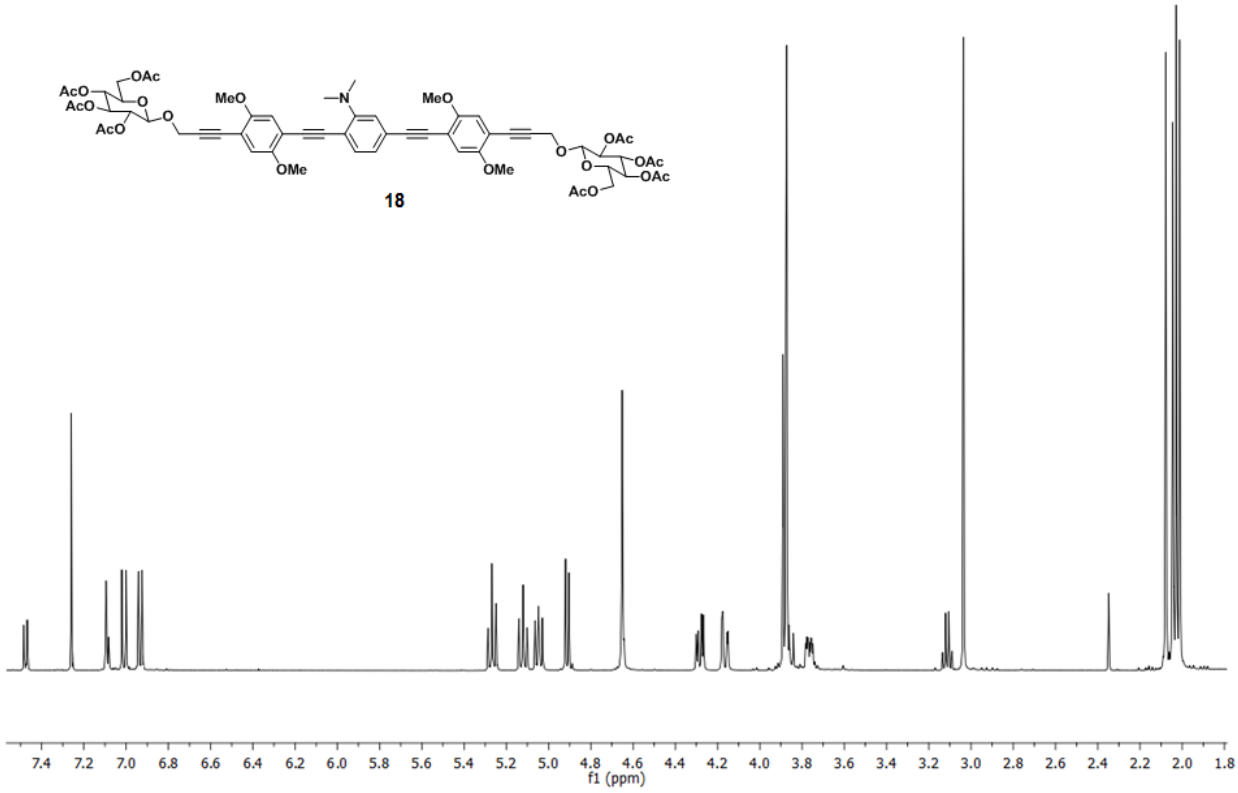

Me
N
Me
TMS
TMS
16
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

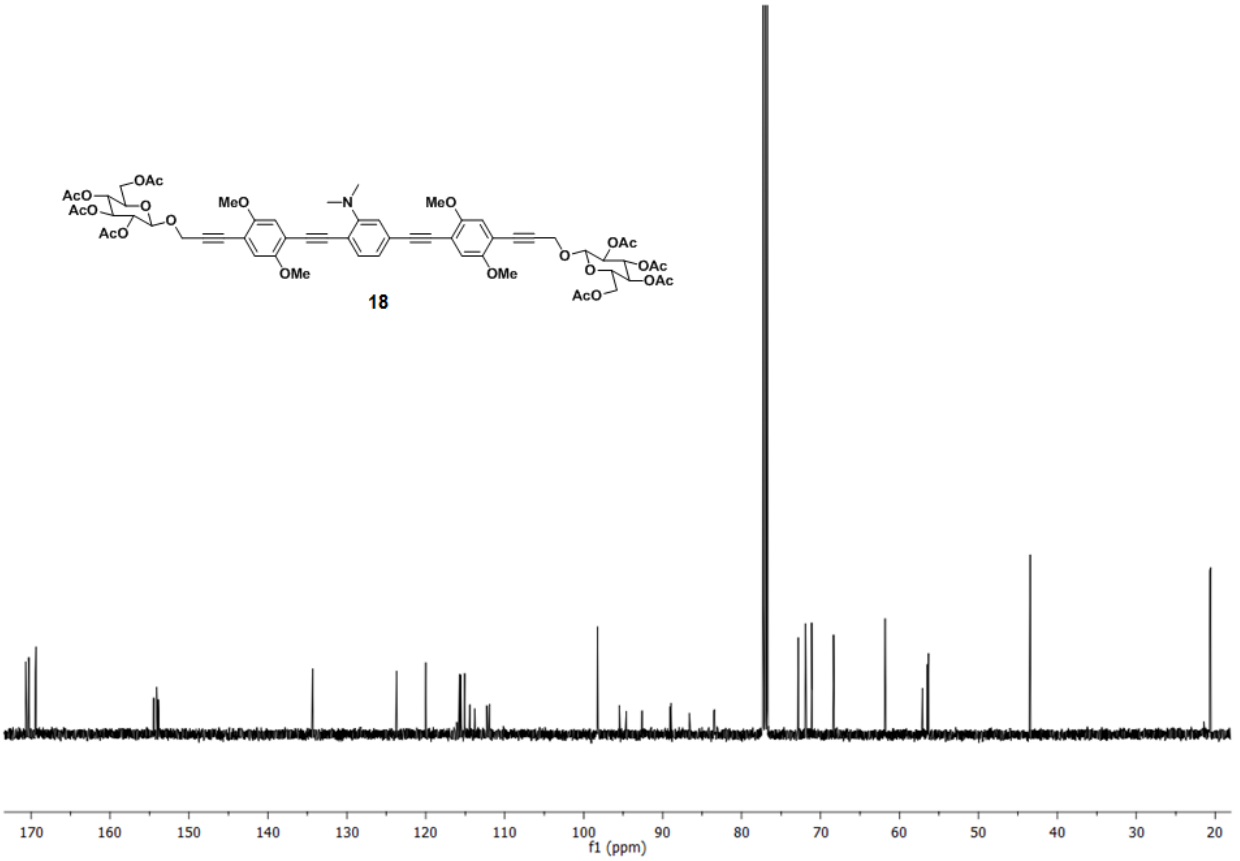

N
TMS
TMS
NO2
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

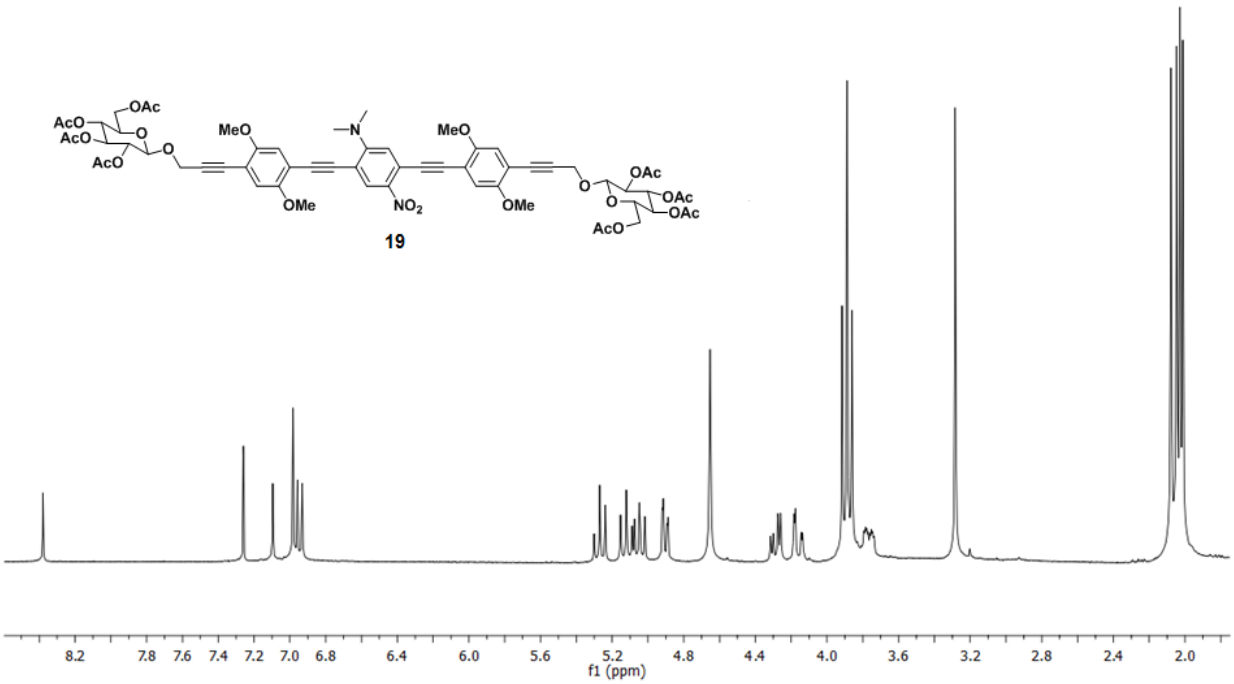
17

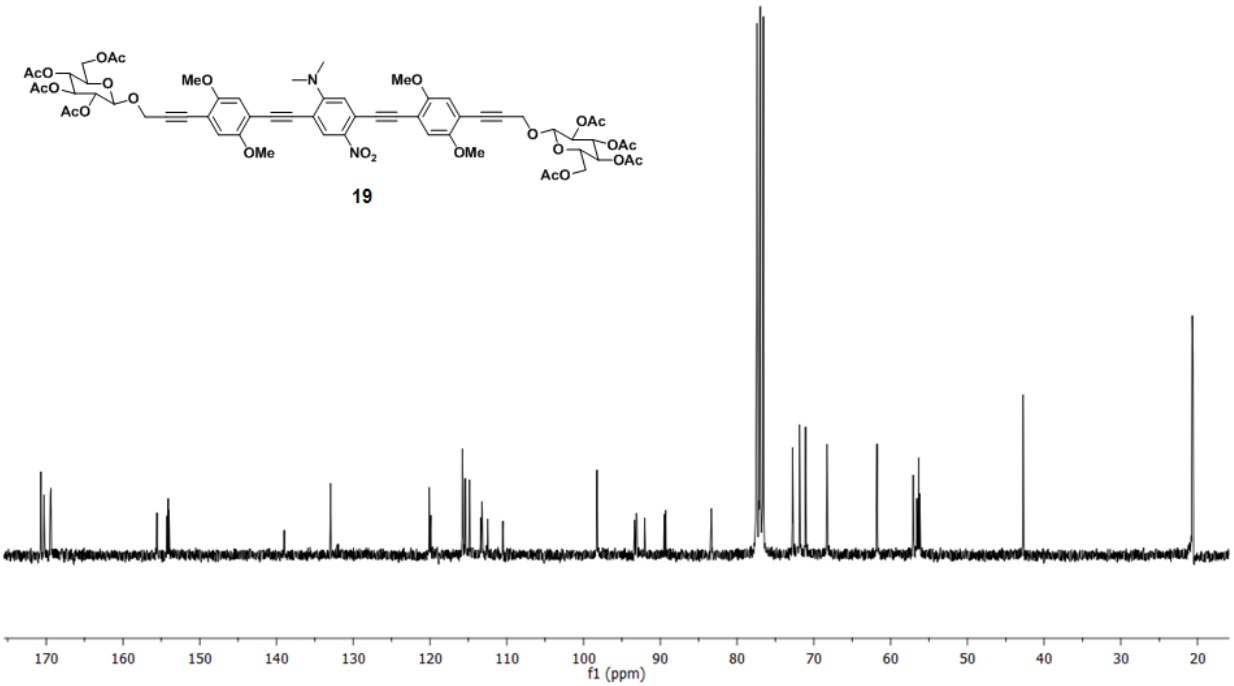

N
TMS
TMS
NO2
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

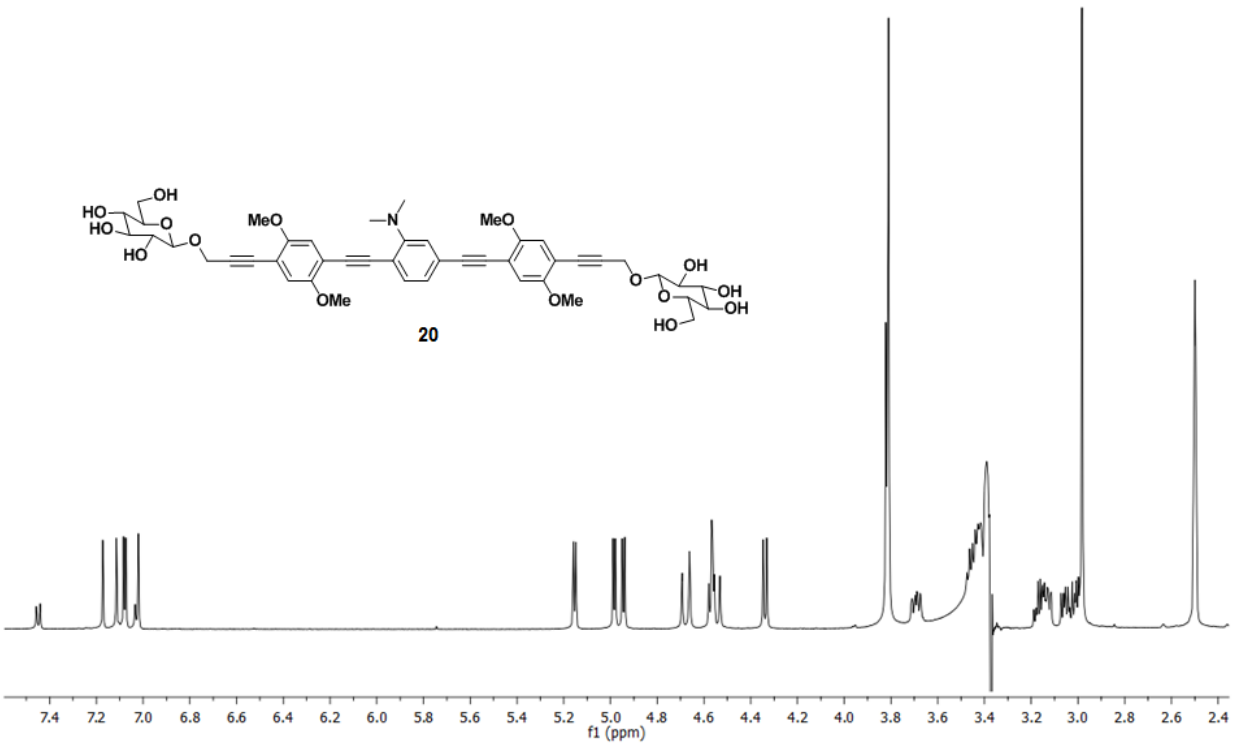
17

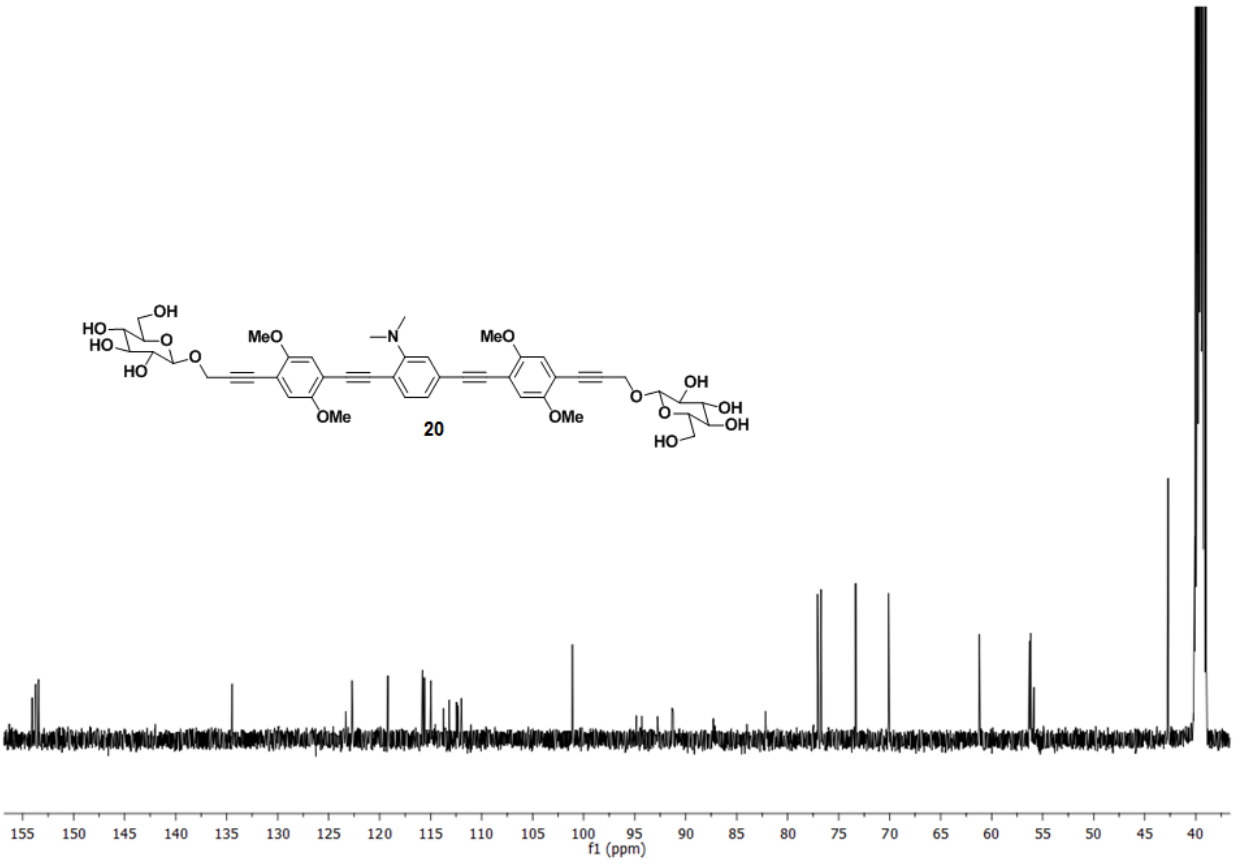

OAc
AcO
AcO
AcO
O
MeO
N
MeO
OMe
OMe
OAc
OAc
OAc
AcO
18
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
f1 (ppm)

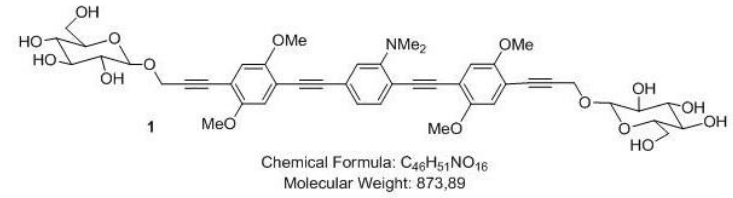

OAc
AcO
AcO
AcO
O
MeO
N
MeO
OMe
OMe
OAc
OAc
OAc
AcO
18
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)


19
8.2
7.8
7.6
7.4
7.2
7.0
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
f1 (ppm)


19
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


HO
HO
HO
OH
O
O
MeO
OMe
N
MeO
OMe
O
OH
OH
OH
HO
20
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4
f1 (ppm)

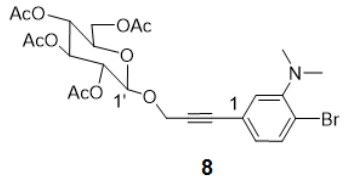

HO
HO
HO
OH
O
O
MeO
OMe
N
MeO
OMe
O
OH
OH
OH
HO
20
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40
f1 (ppm)



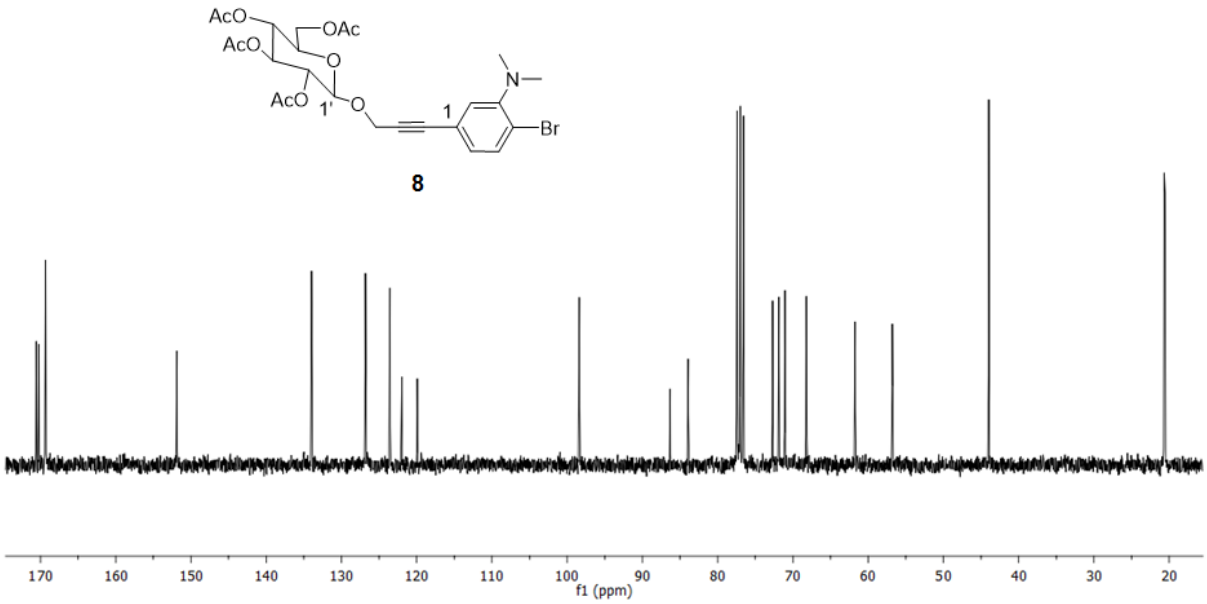
Chemical Formula: $C_{46}H_{51}NO_{16}$
Molecular Weight: 873,89

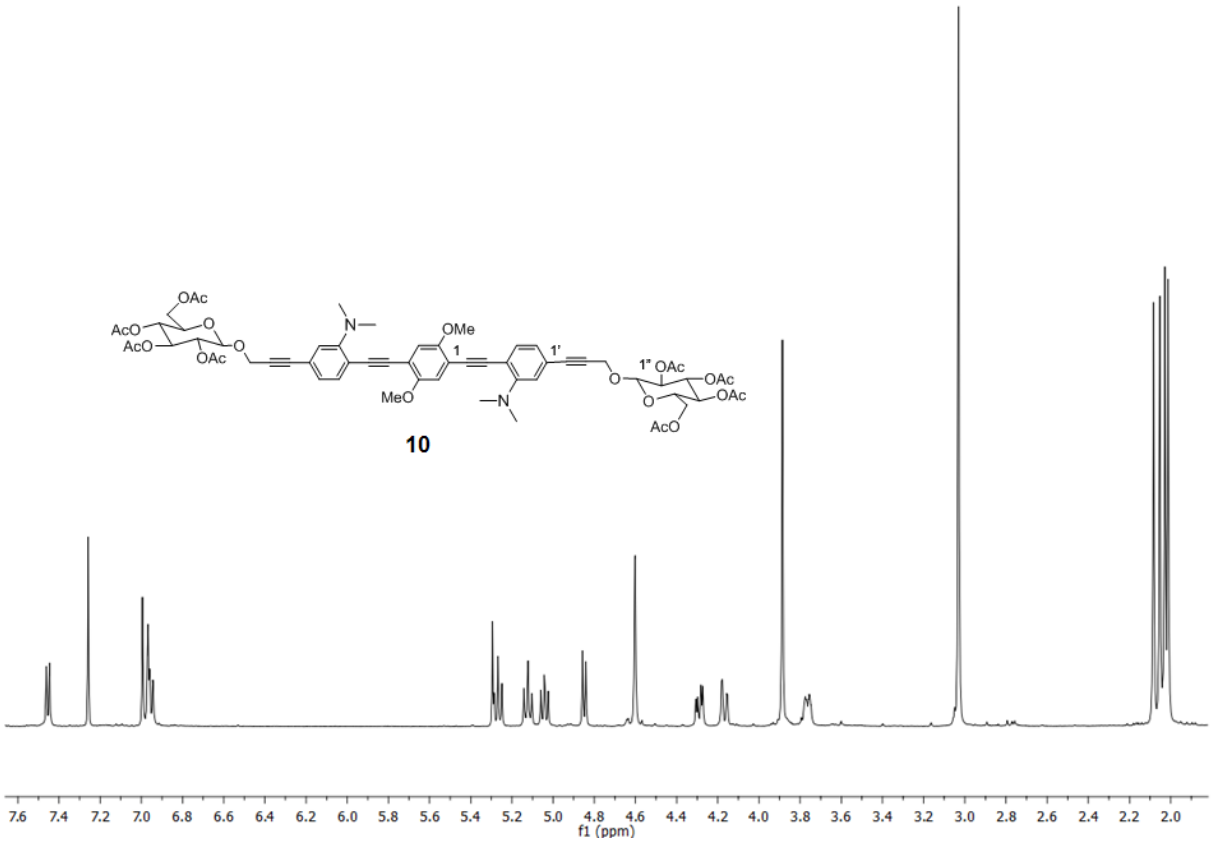
Average Purity 96.3%

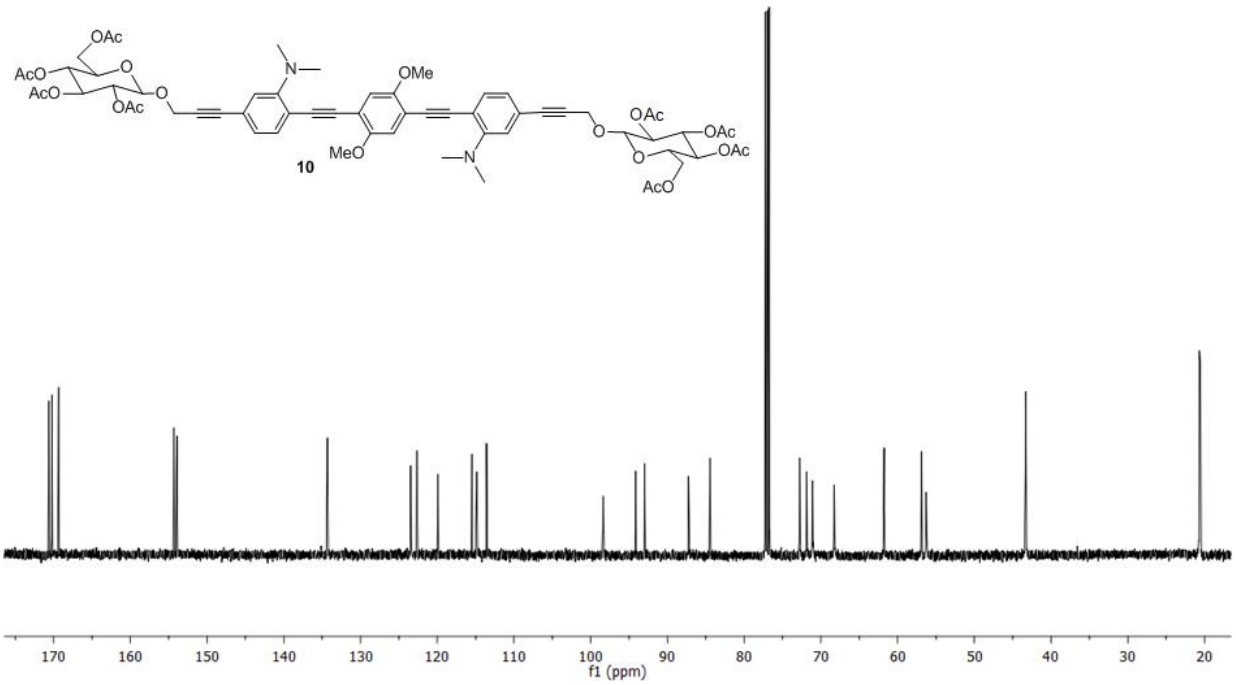
Assuming sample weight: 2.02mg (dissolved in 0.60mL of *dmso-d6*), and M.W.=873.89
Using reference Compound Maleic acid TraceCERT® :1.96mg (dissolved in 0.58mL of *dmso-d6*), M.W.=116.07
○ Sample integral: 77.18 (6 nuclides)
△ Reference integral: 22.82 (2 nuclides)

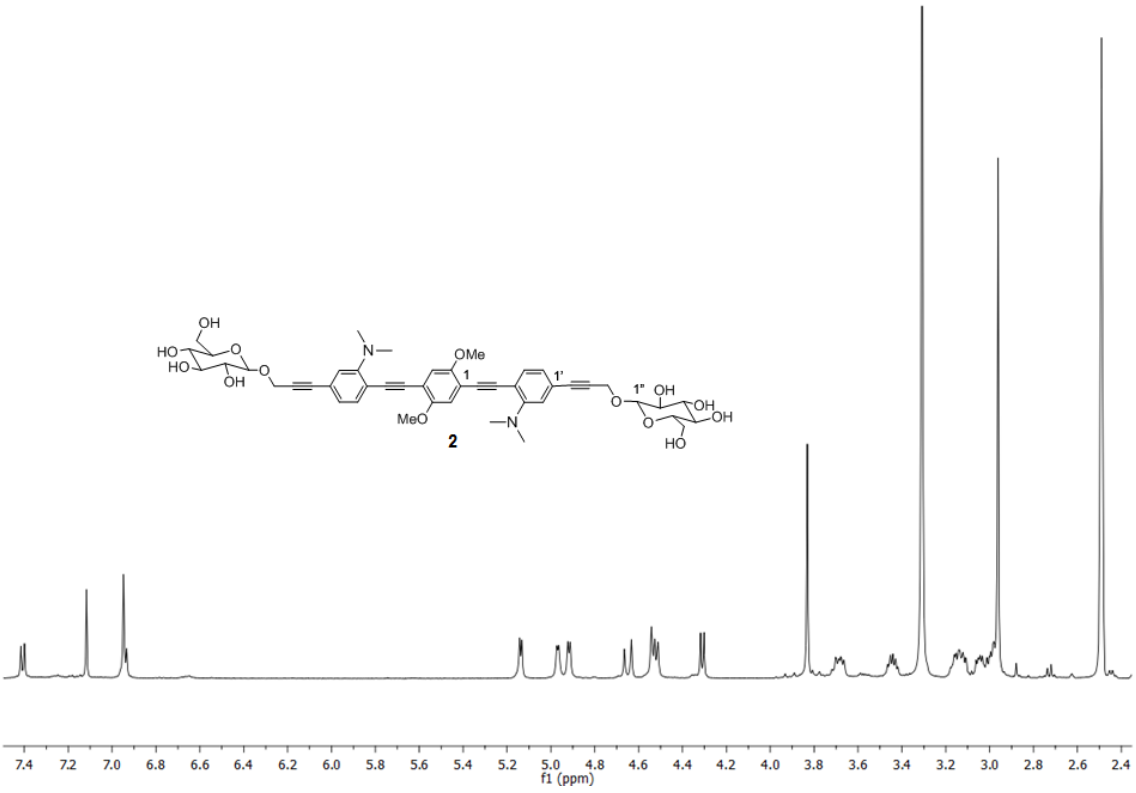


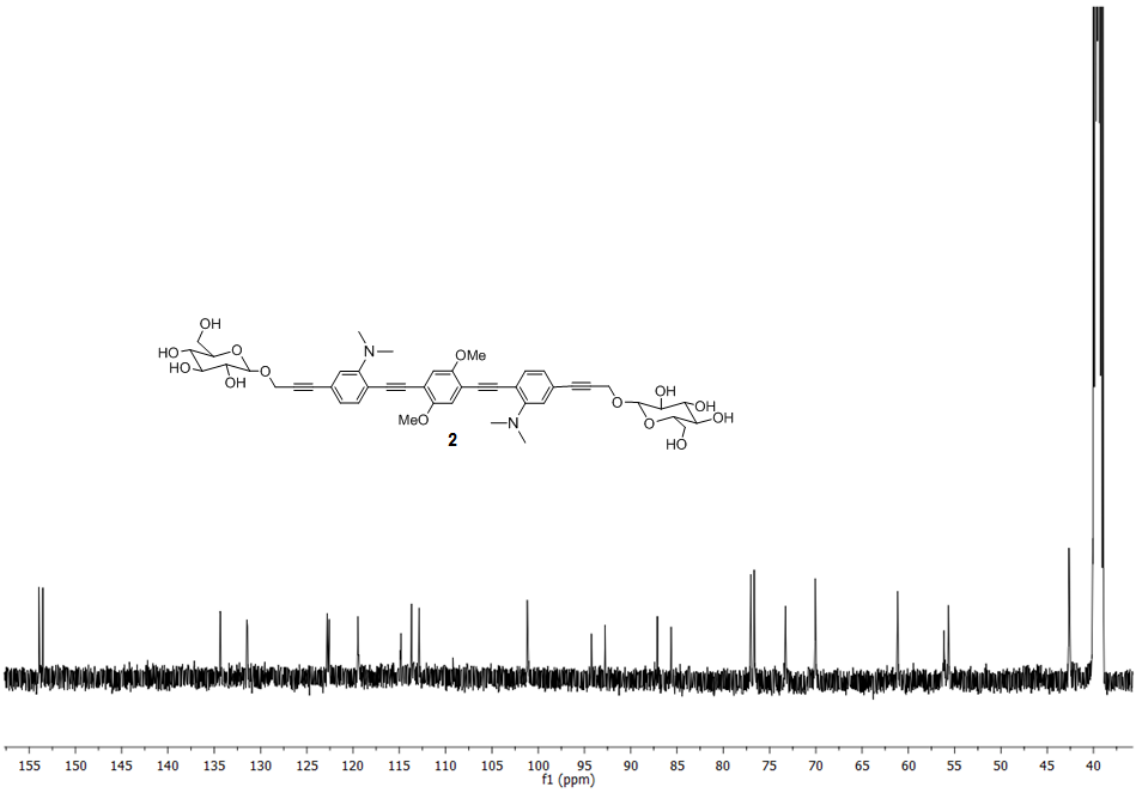
8

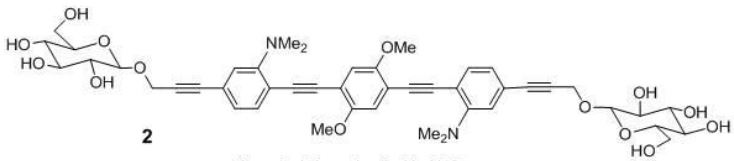

AcO
OAc
AcO
O
AcO
1'
O
1
N
Br
8
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


OAc
AcO
AcO
O
OAc
O
N
OMe
1
1'
MeO
N
1" OAc
OAc
OAc
O
AcO
10
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
f1 (ppm)


OAc
AcO
AcO
OAc
O
O
N
OMe
MeO
N
OAc
OAc
OAc
O
O
AcO
10
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


OH
HO
HO
OH
O
O
N
OMe
1
1'
MeO
N
1"
OH
OH
OH
O
HO
2
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
f1 (ppm)

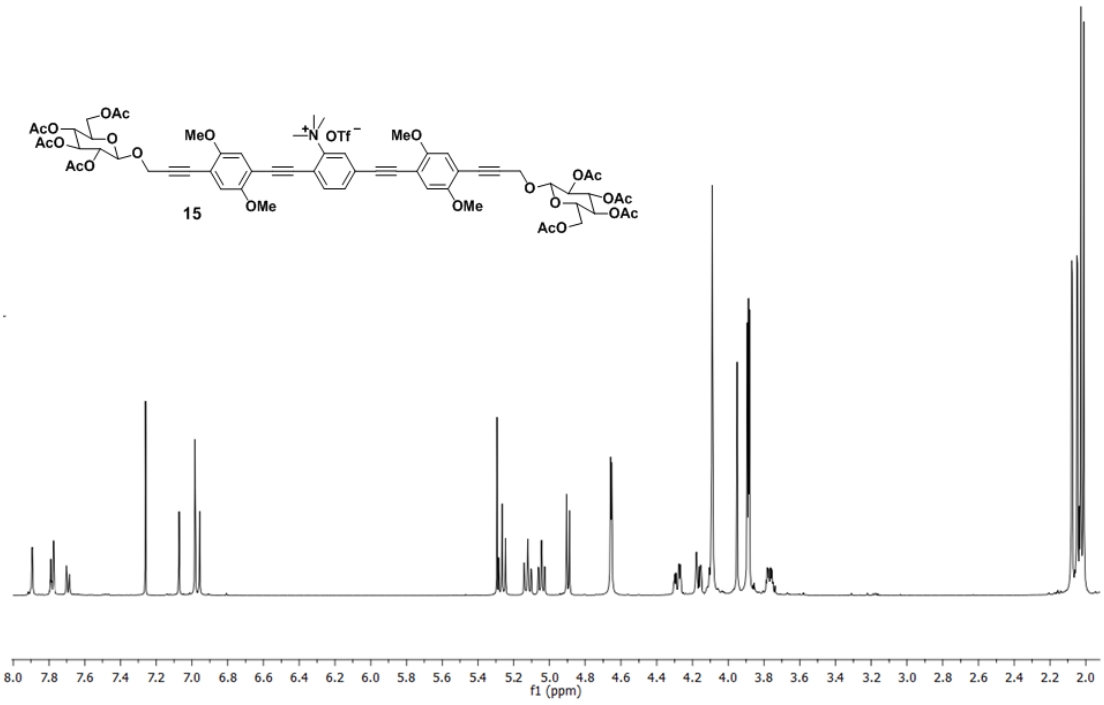


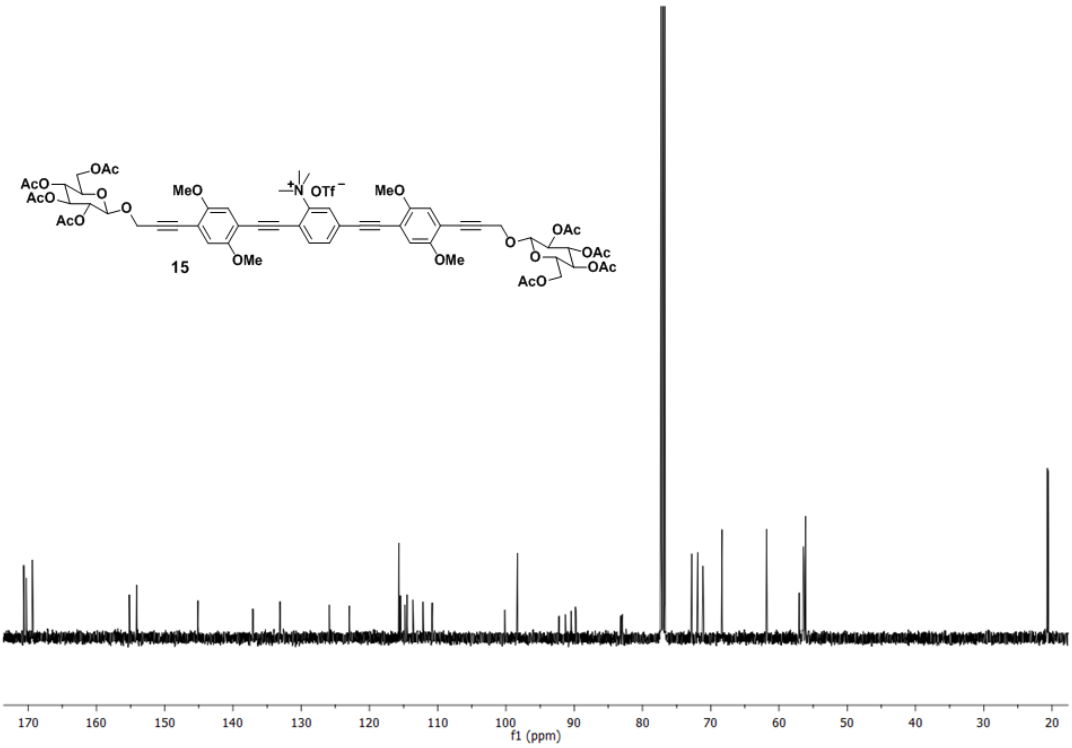


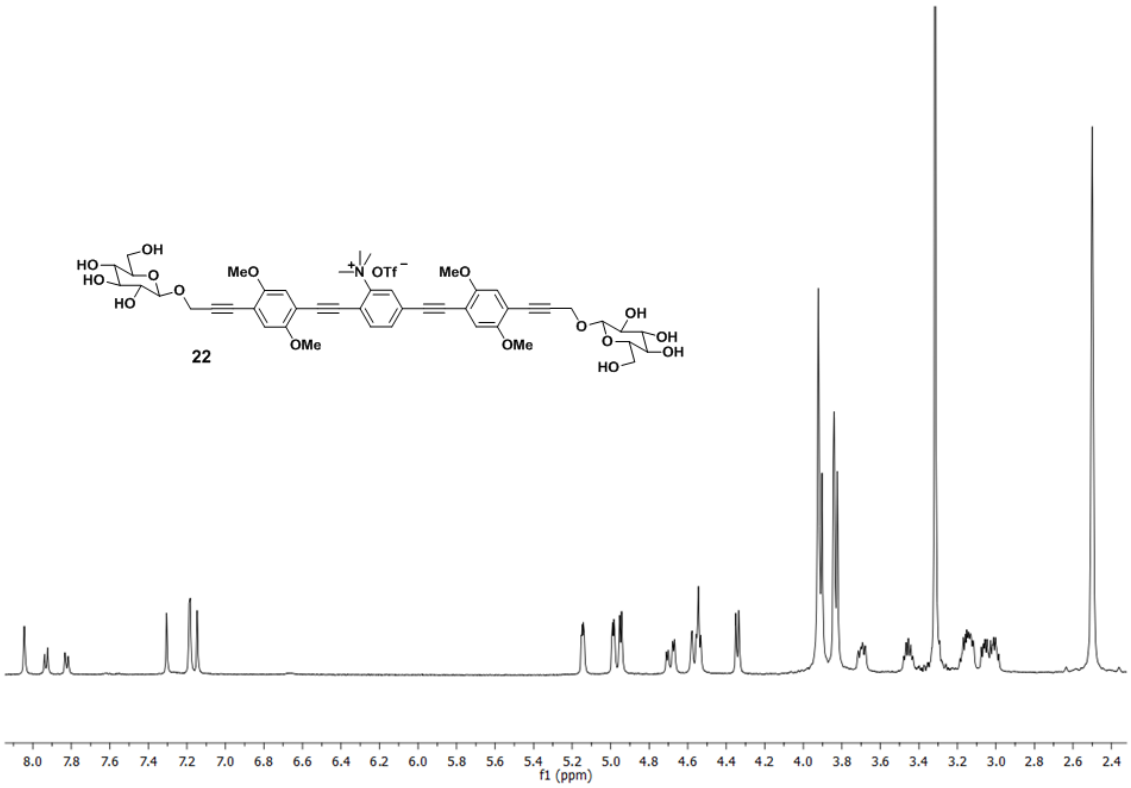
Chemical Formula: $C_{46}H_{52}N_2O_{14}$
Molecular Weight: 856,91

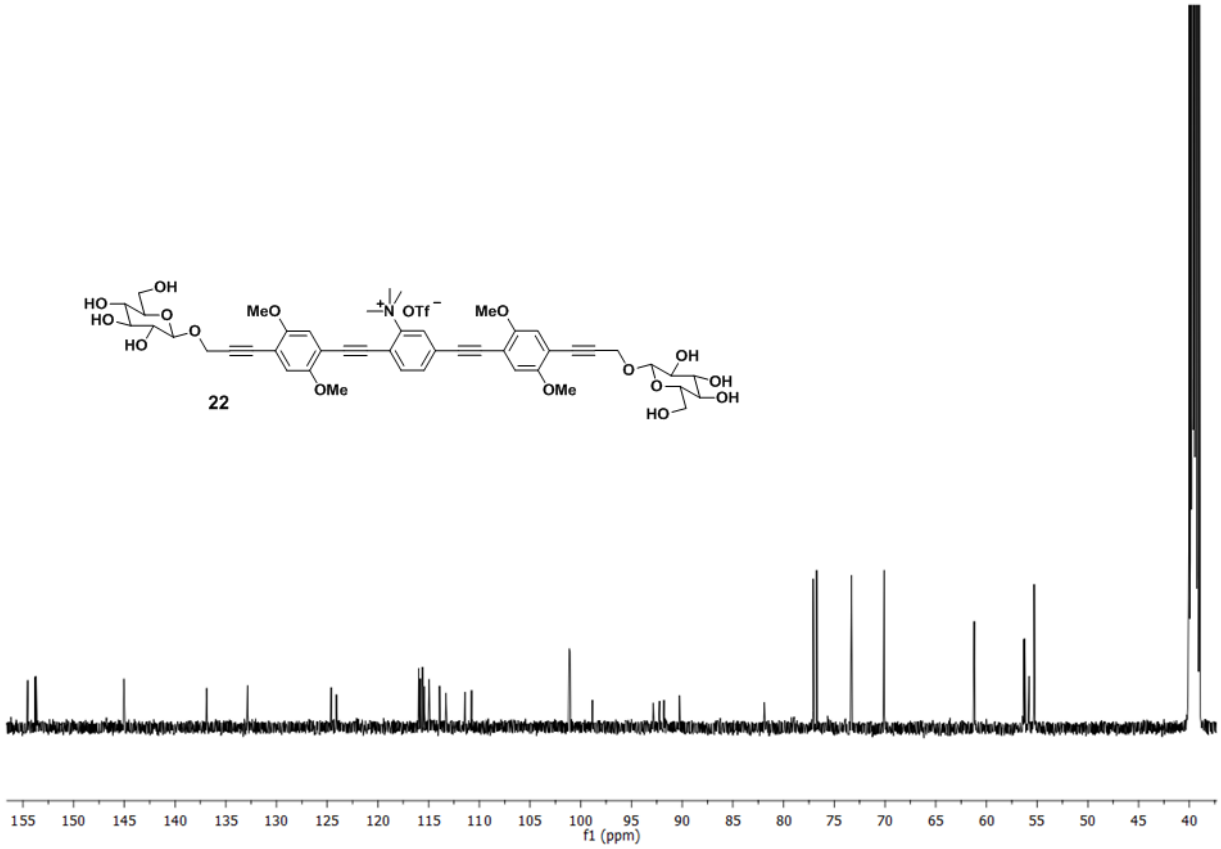
Average Purity 96.3%

Assuming sample weight: 2.52mg (dissolved in 0.60mL of *dmso-d6*), and Mol Weight=856.91
Using reference Compound Maleic acid TraceCERT®: 1.96mg (dissolved in 0.58mL of *dmso-d6*), Mol weight=116.07
○ Sample integral: 81.15 (6 nuclides)
△ Reference integral: 18.85 (2 nuclides)


OAc
AcO
AcO
AcO
O
O
MeO
OMe
+N
OTf
−
MeO
OMe
O
OAc
OAc
OAc
O
AcO
15
8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0
f1 (ppm)


AcO
AcO
AcO
OAc
O
MeO
OMe
15
OTf
MeO
OMe
O
OAc
OAc
OAc
O
AcO
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


HO
HO
HO
OH
O
O
MeO
OMe
+N
OTf
-
MeO
OMe
O
OH
OH
O
HO
22
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
f1 (ppm)


HO
HO
HO
OH
O
O
MeO
OMe
+N
OTf
−
MeO
OMe
O
OH
OH
OH
O
HO
22
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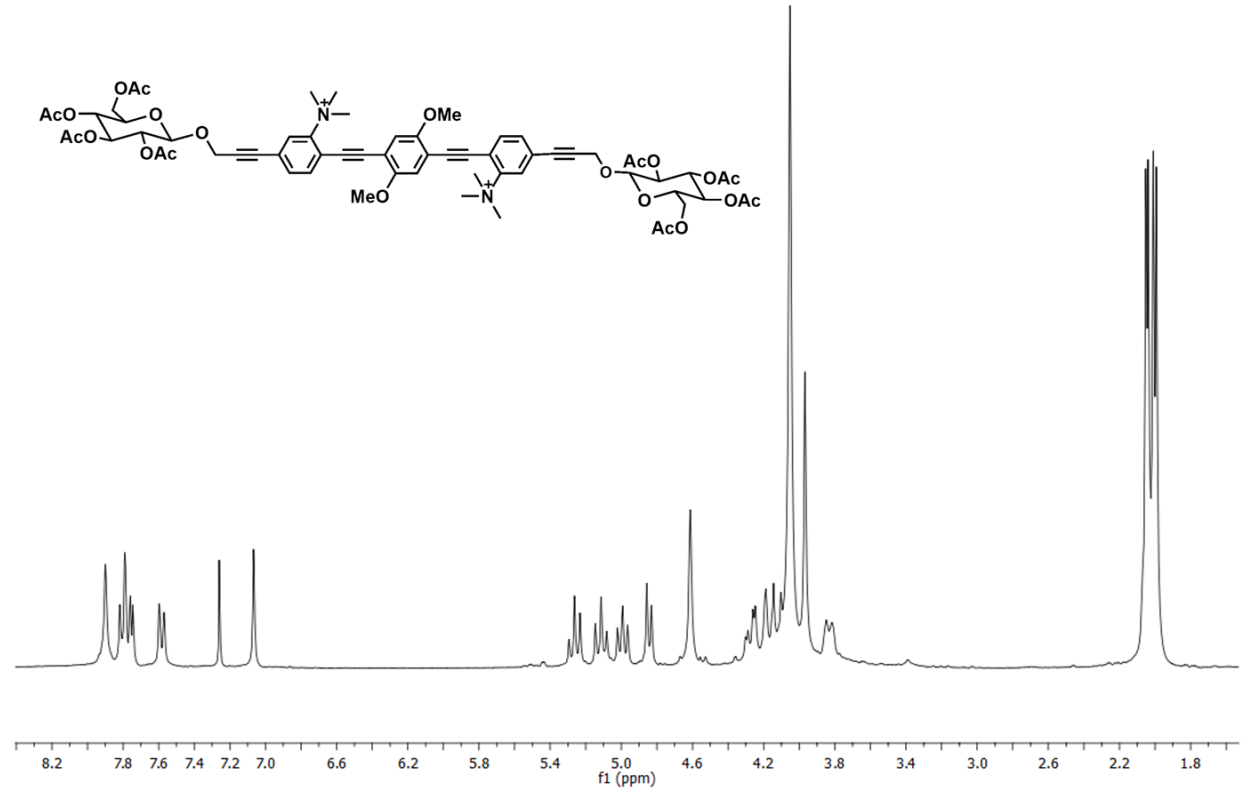
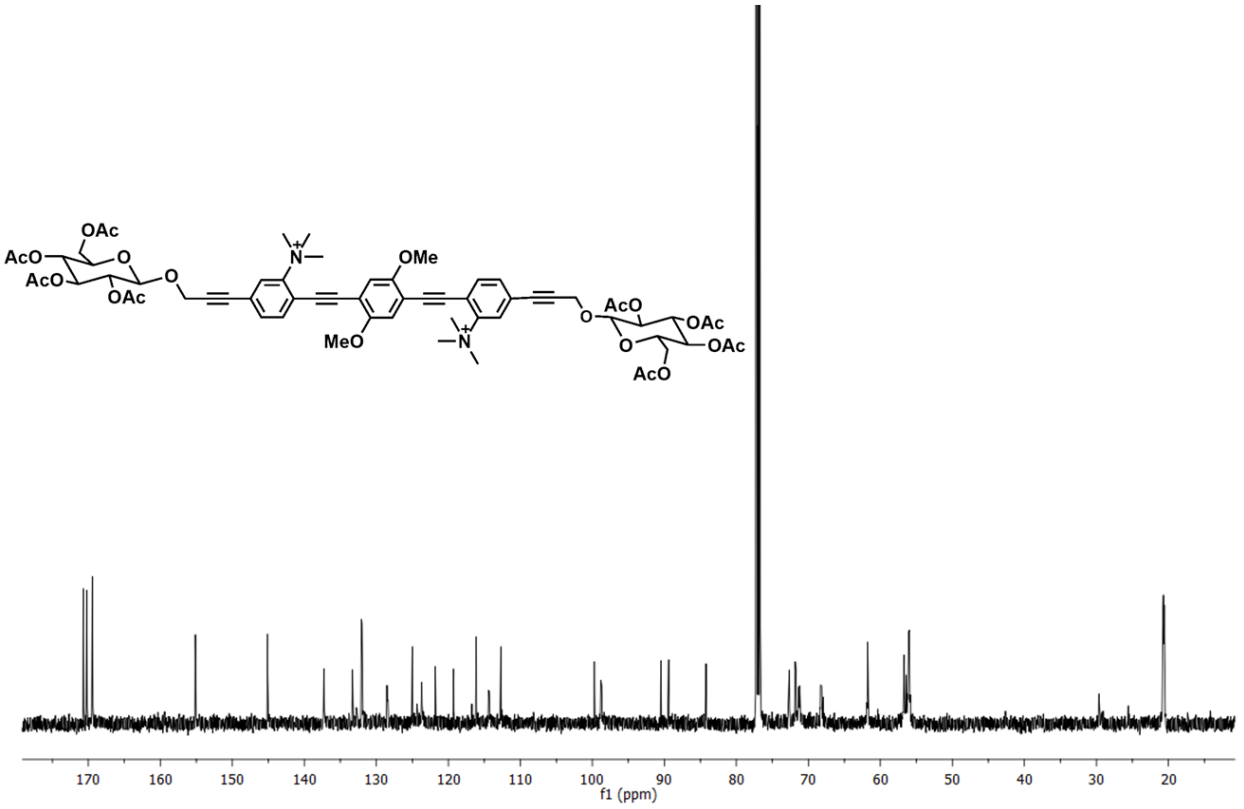
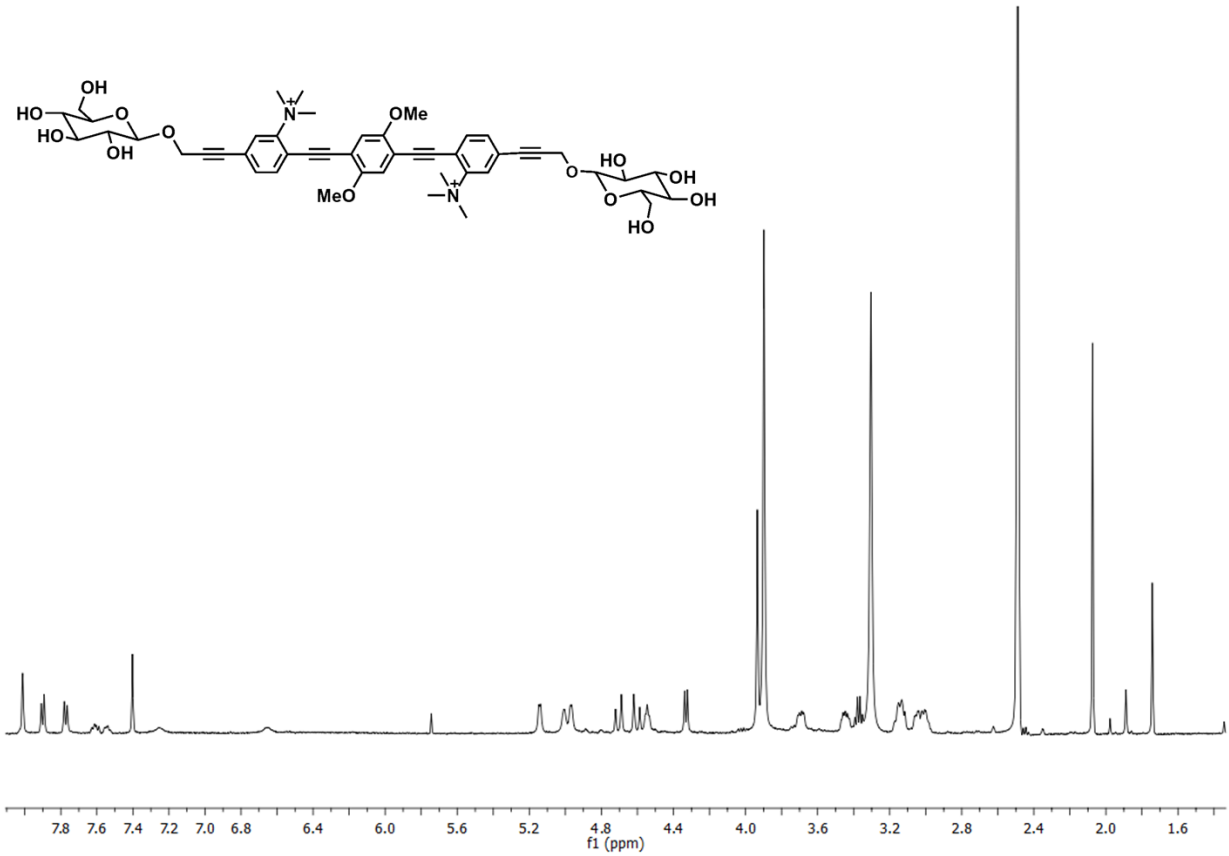
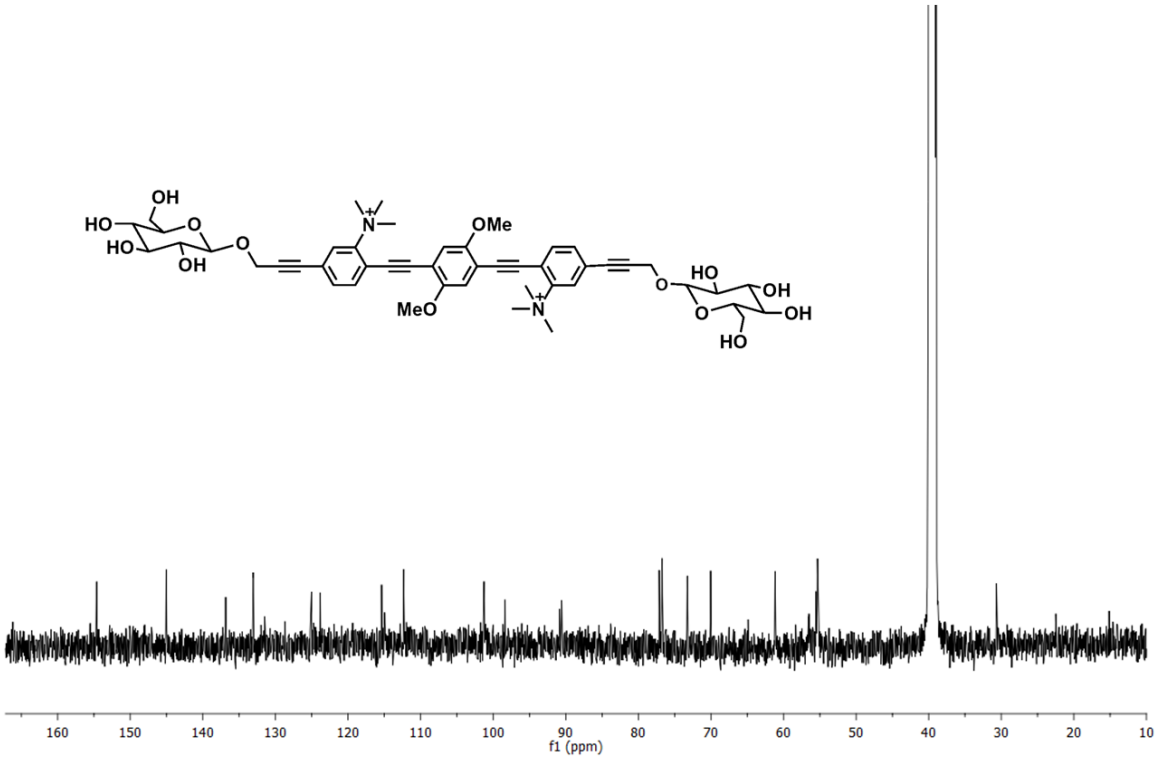
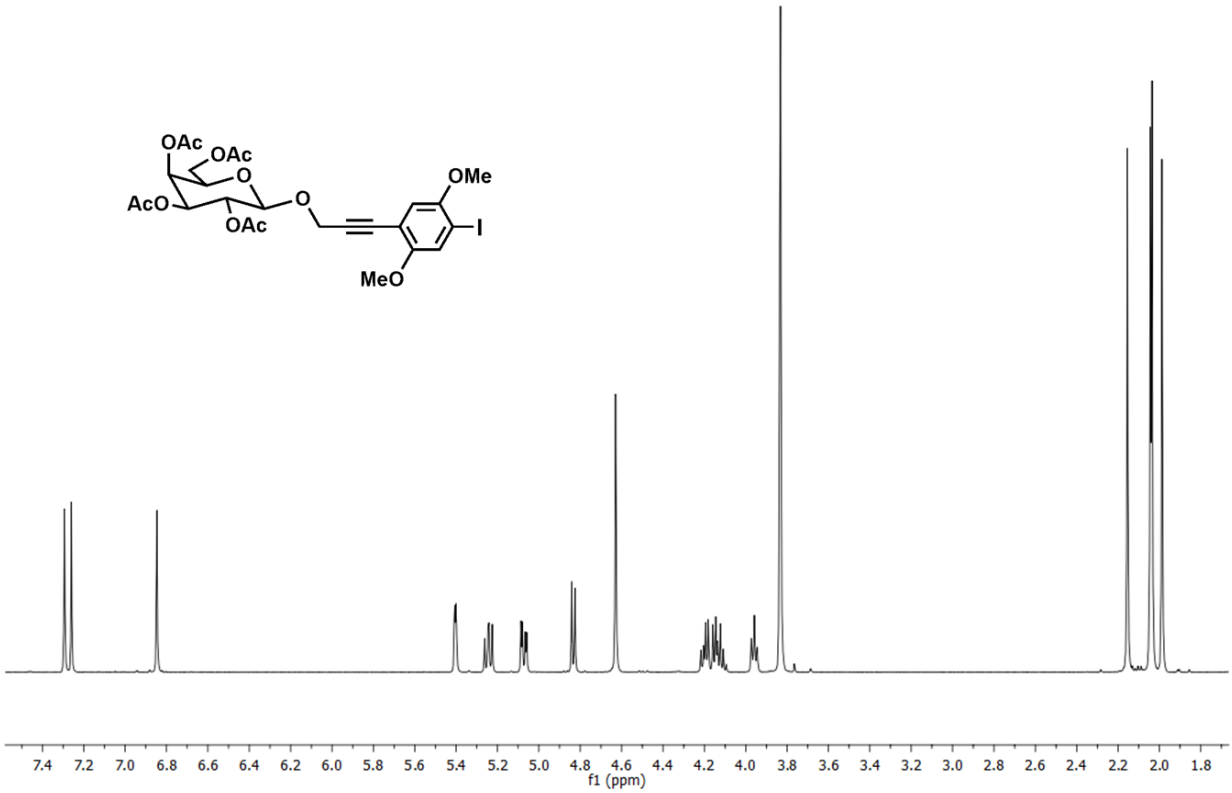
f1 (ppm)

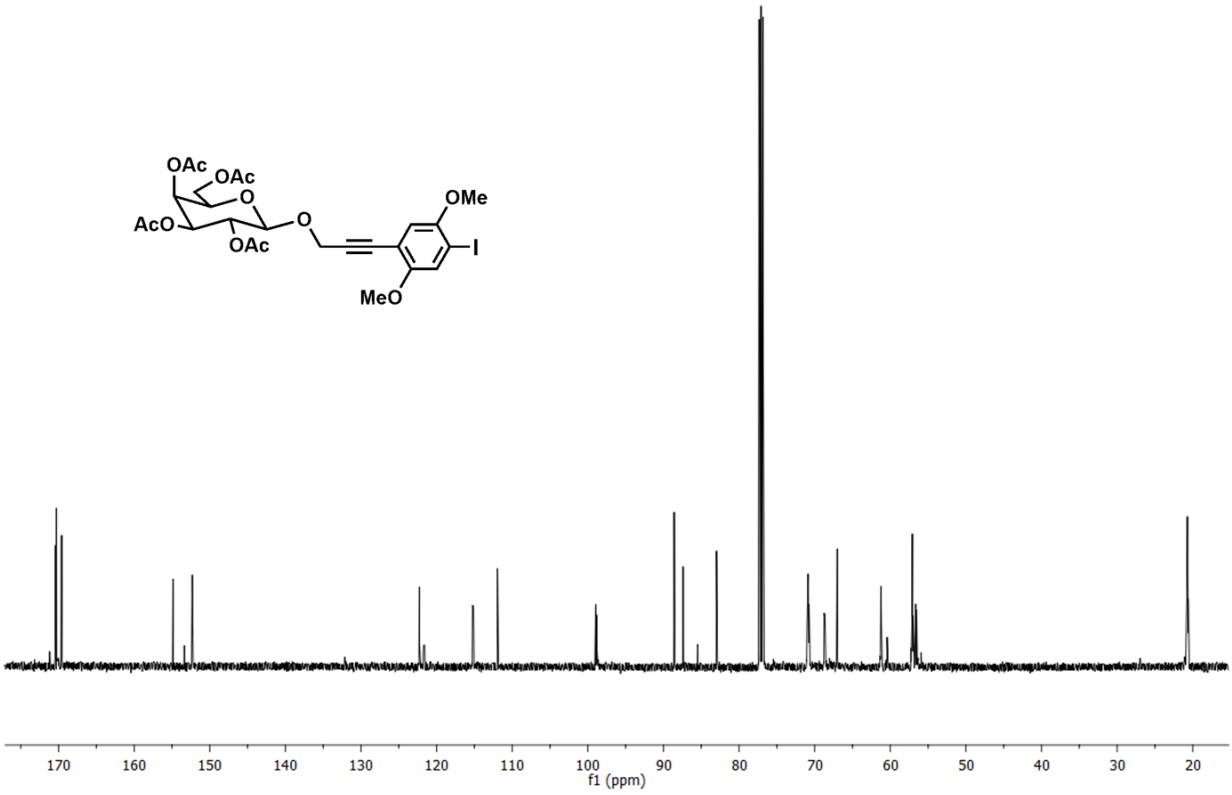

OAc
AcO
AcO
OAc
O
OMe
MeO
AcO
OAc
OAc
AcO
8.2
7.8
7.6
7.4
7.2
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
1.8
f1 (ppm)

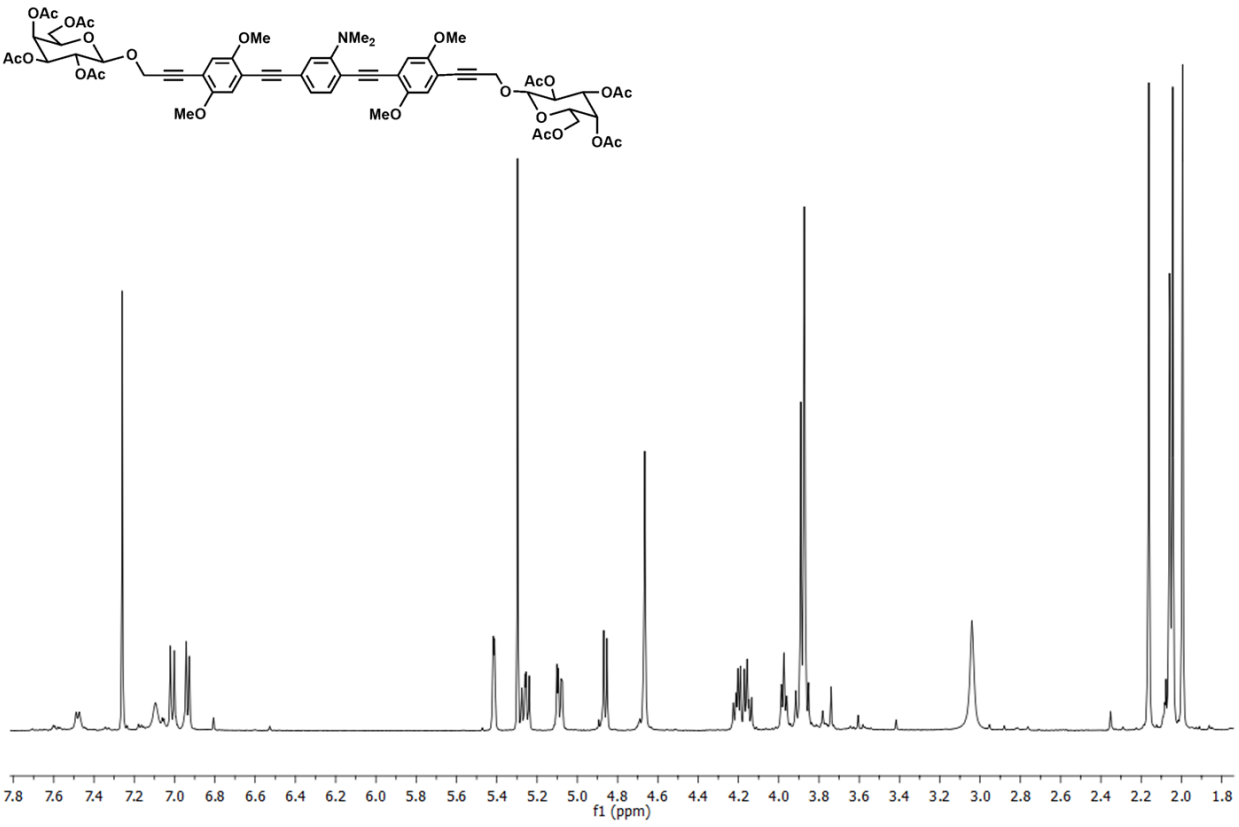

OAc
AcO
AcO
OAc
O
OMe
MeO
AcO
OAc
OAc
AcO
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

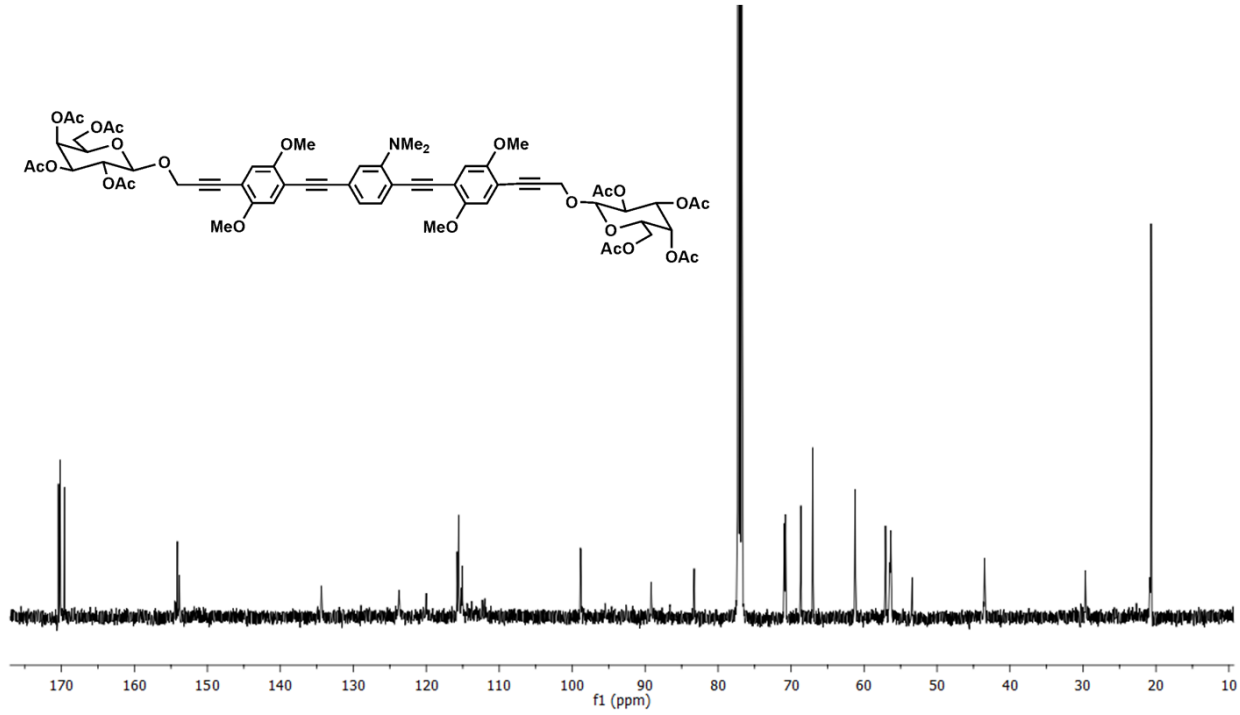

OH
HO
HO
OH
O
OMe
MeO
HO
OH
OH
HO
7.8 7.6 7.4 7.2 7.0 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6
f1 (ppm)

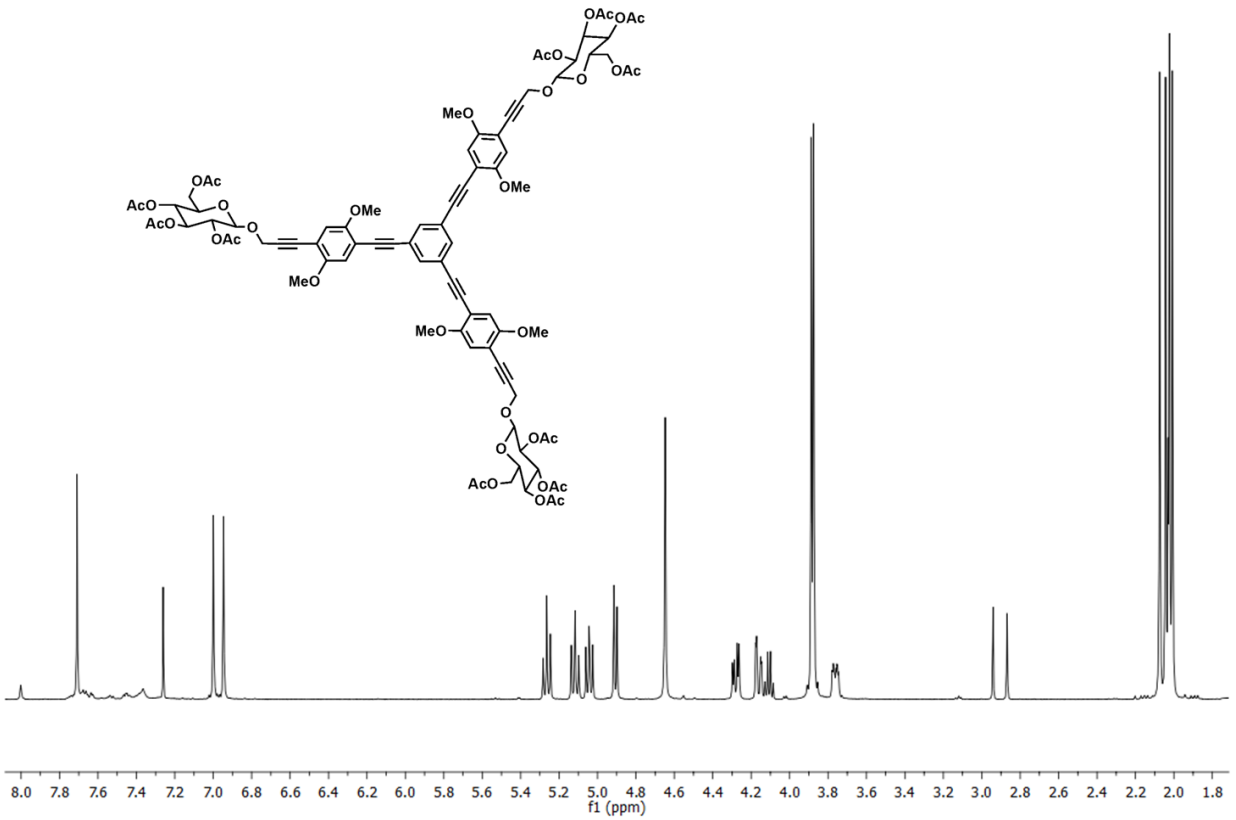

OH
HO
HO
OH
OMe
MeO
HO
OH
OH
HO
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

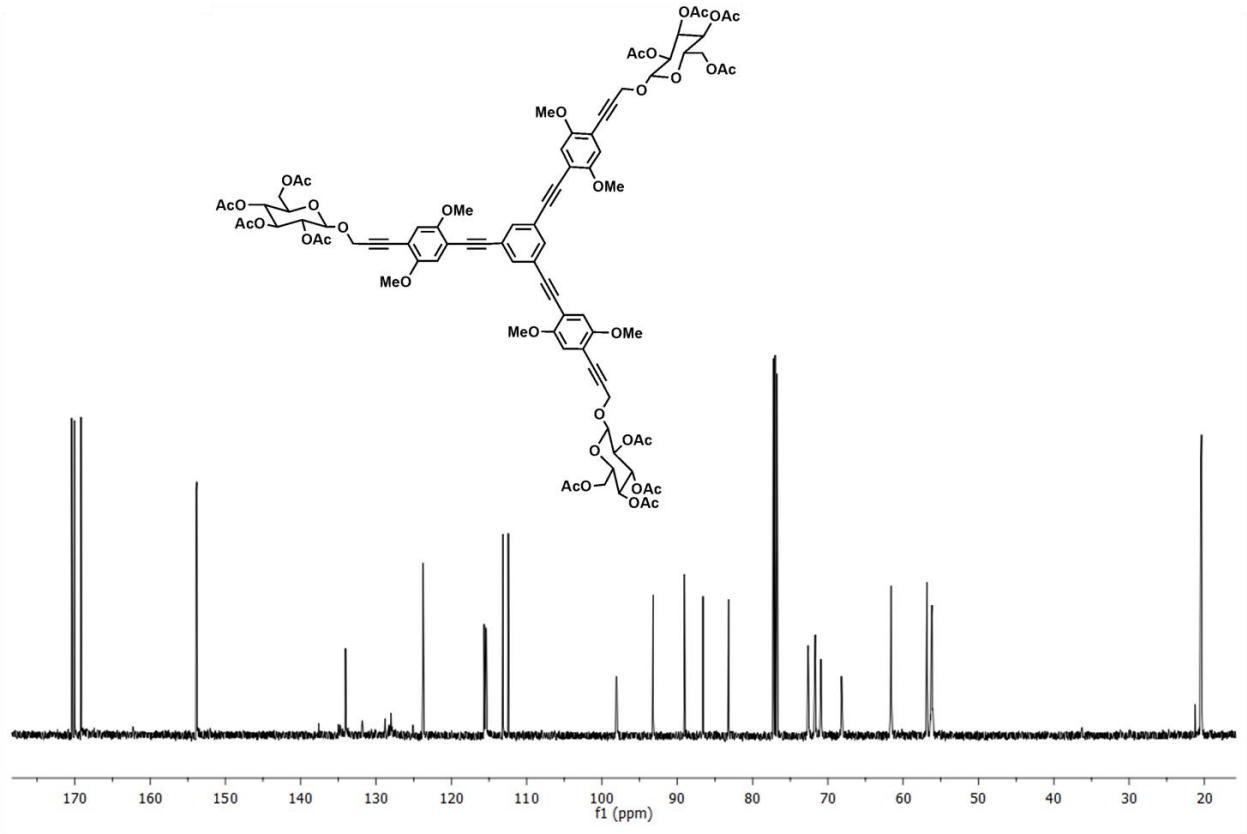

OAc
OAc
O
AcO
OAc
O
OMe
I
MeO
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
f1 (ppm)

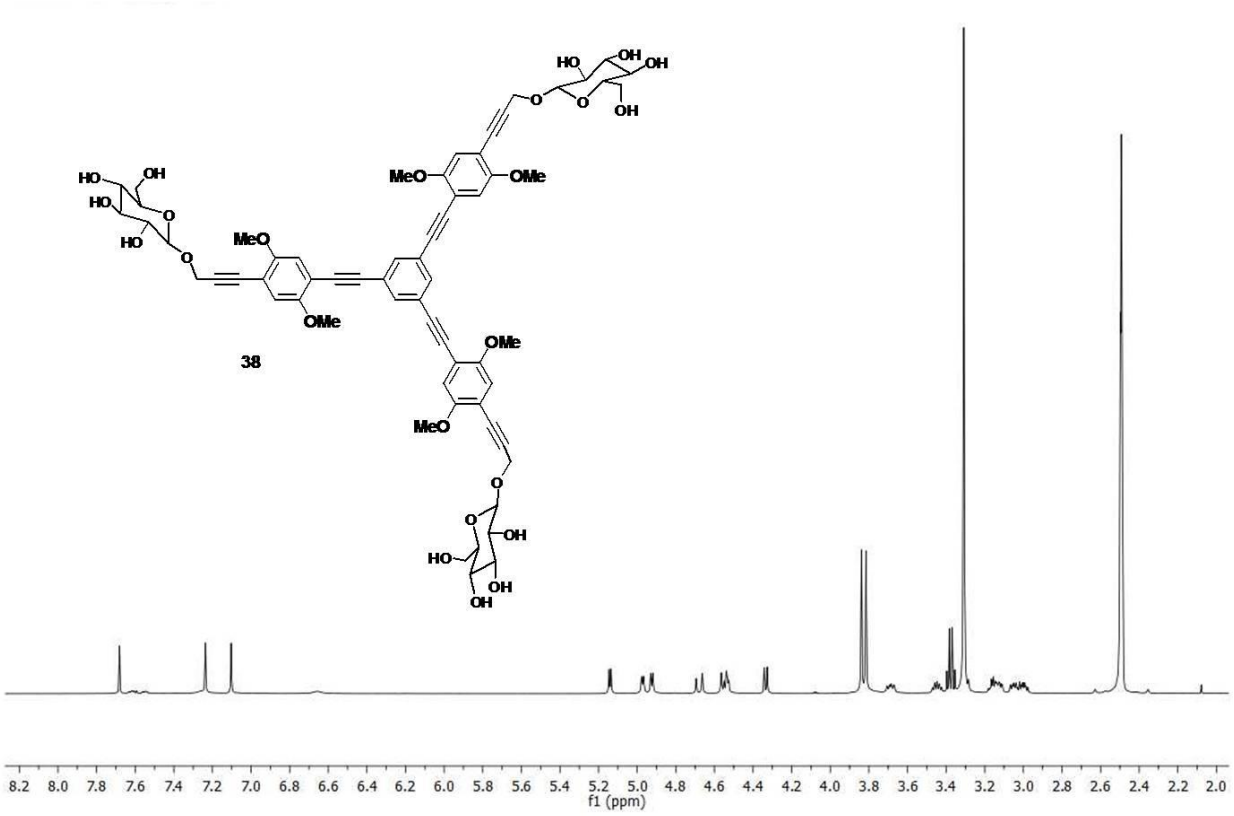

OAc
OAc
O
AcO
OAc
O
OMe
I
MeO
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

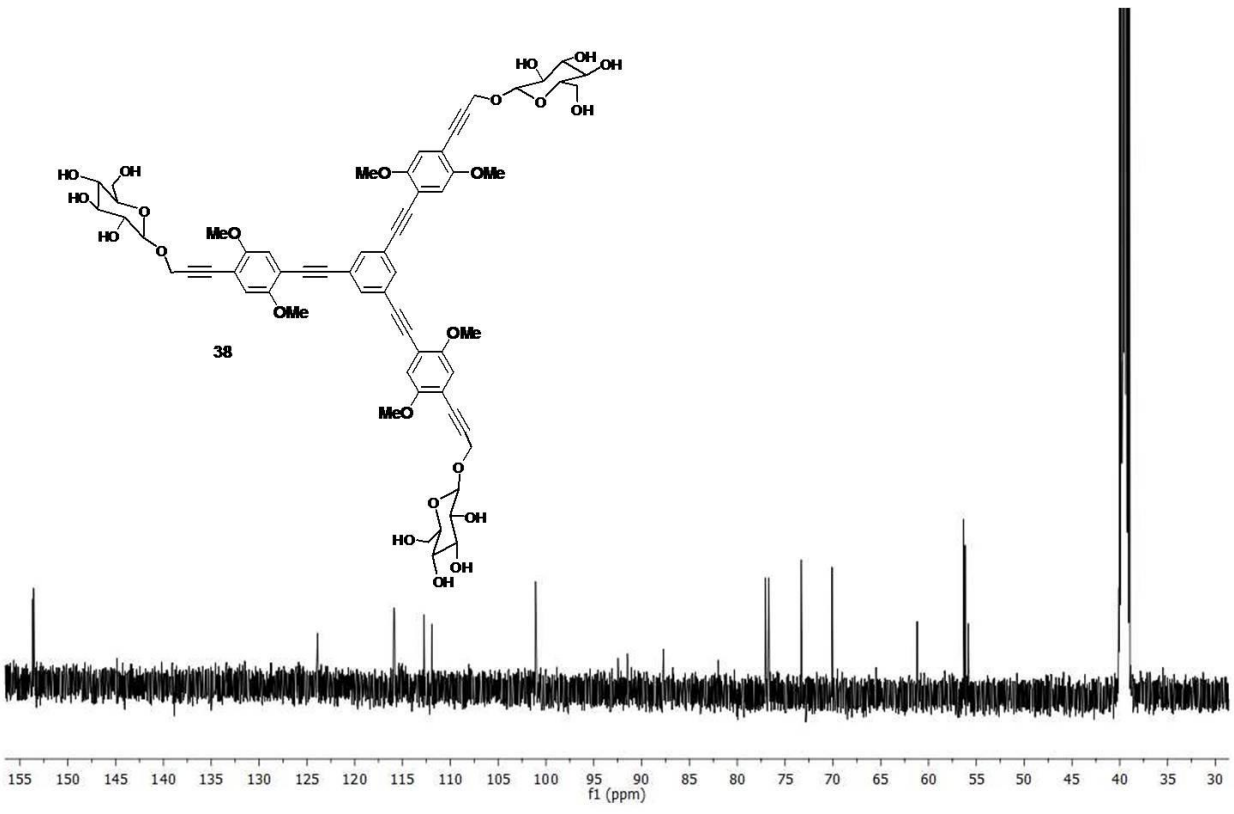

OAc
OAc
AcO
OAc
OMe
NMe2
OMe
MeO
MeO
AcO
OAc
AcO
OAc
7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
f1 (ppm)

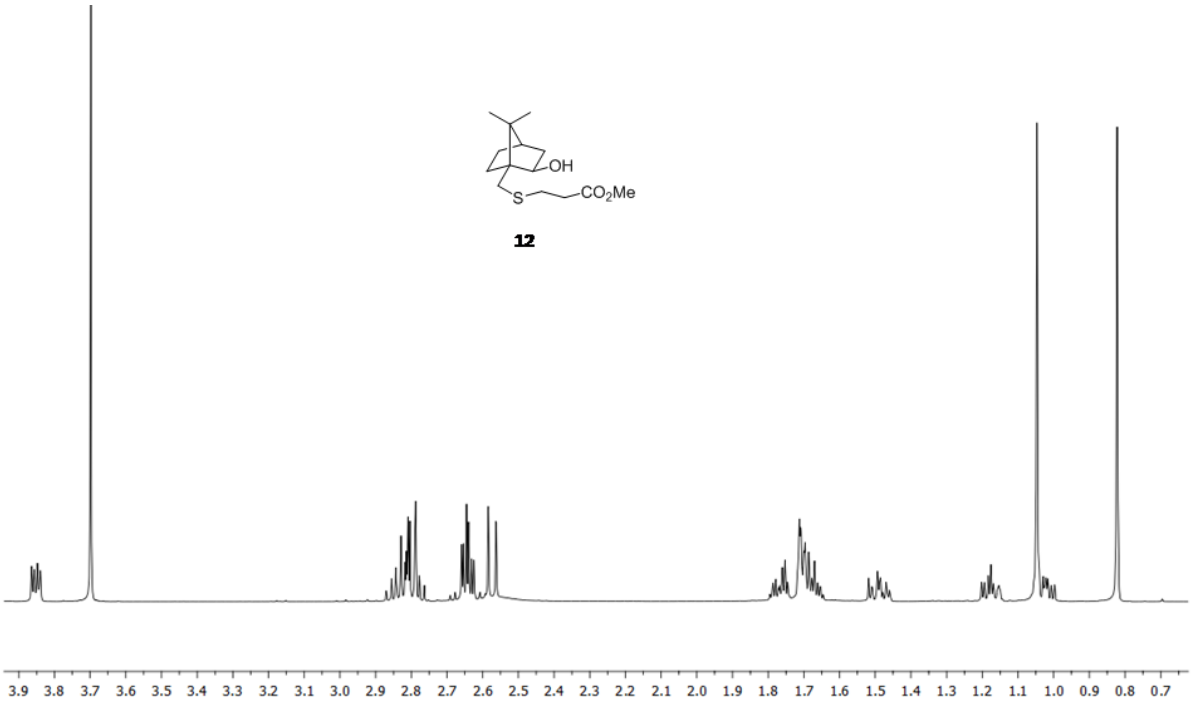

OAc
OAc
AcO
OAc
OMe
NMe2
OMe
MeO
MeO
AcO
OAc
AcO
OAc
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

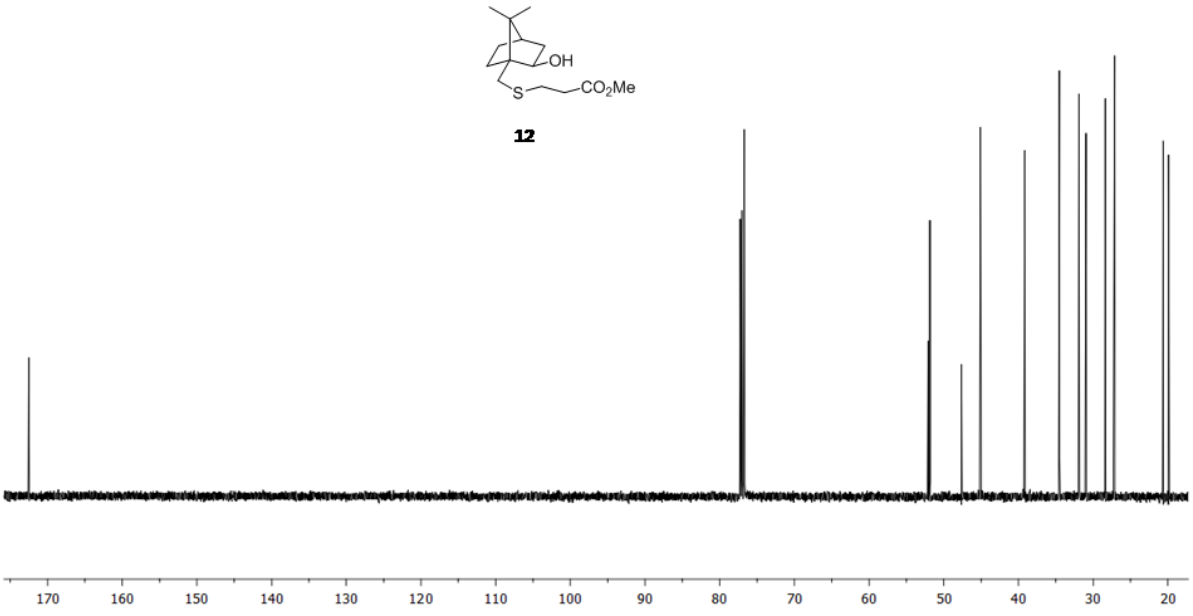

OAc
AcO
AcO
OAc
O
MeO
OMe
f1 (ppm)

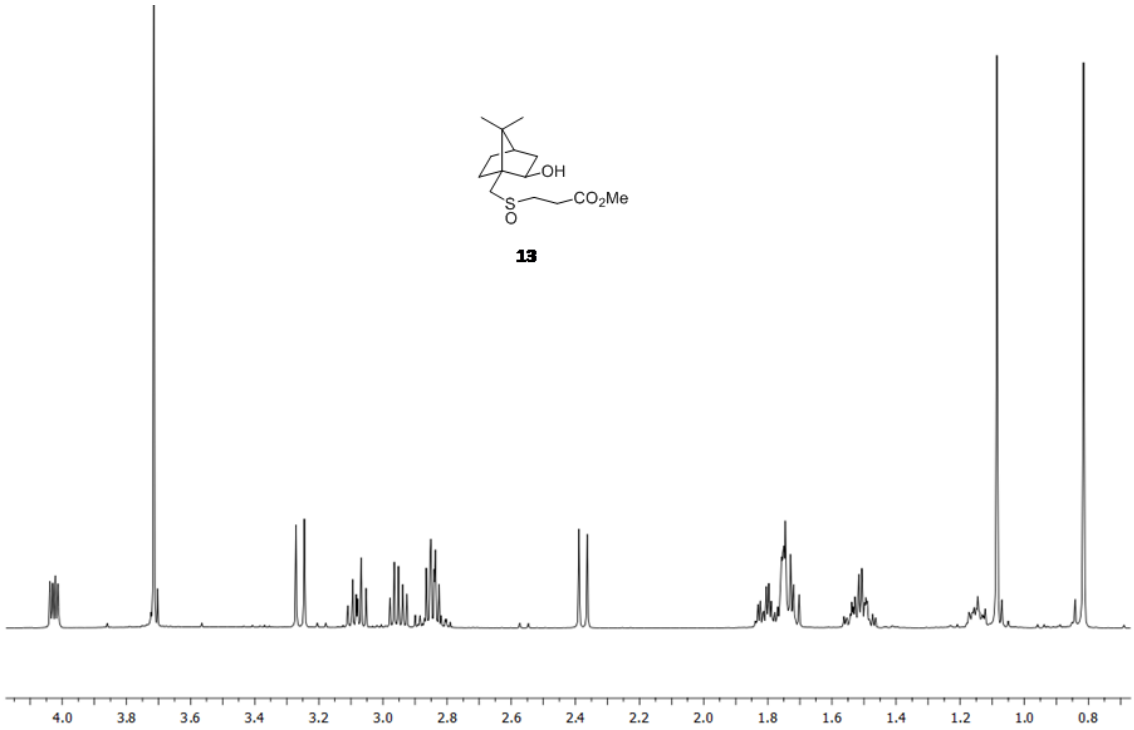

OAc
AcO
AcO
OAc
O
MeO
OMe
f1 (ppm)


38
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0
f1 (ppm)

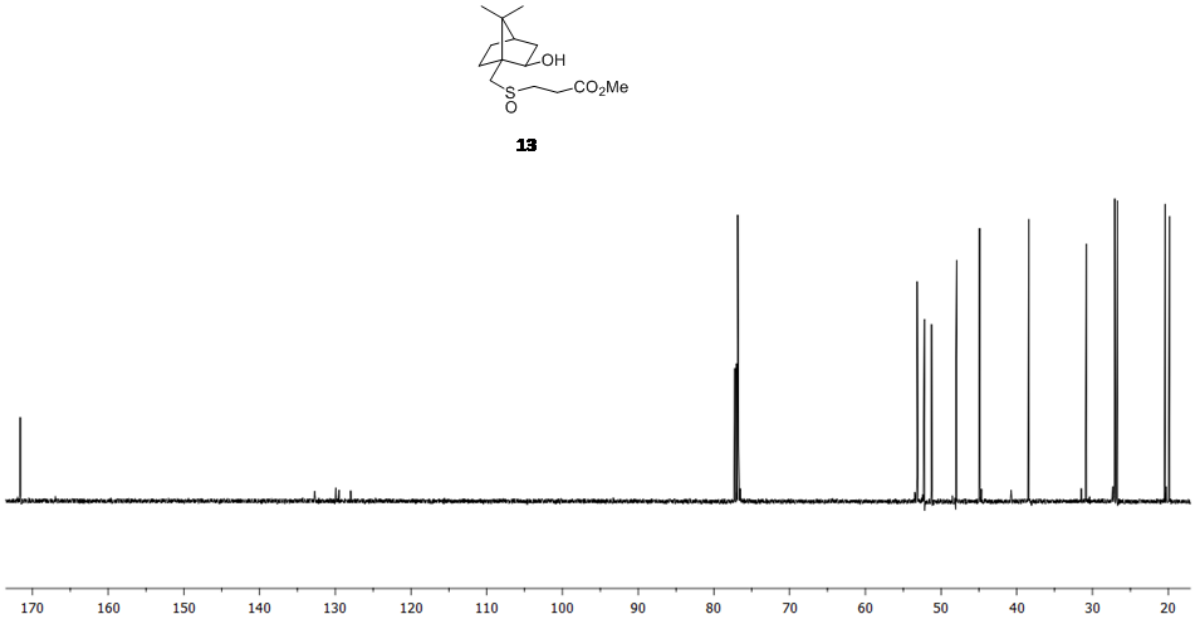

38
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30
f1 (ppm)


OH
S
CO2Me
12
3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8 0.7

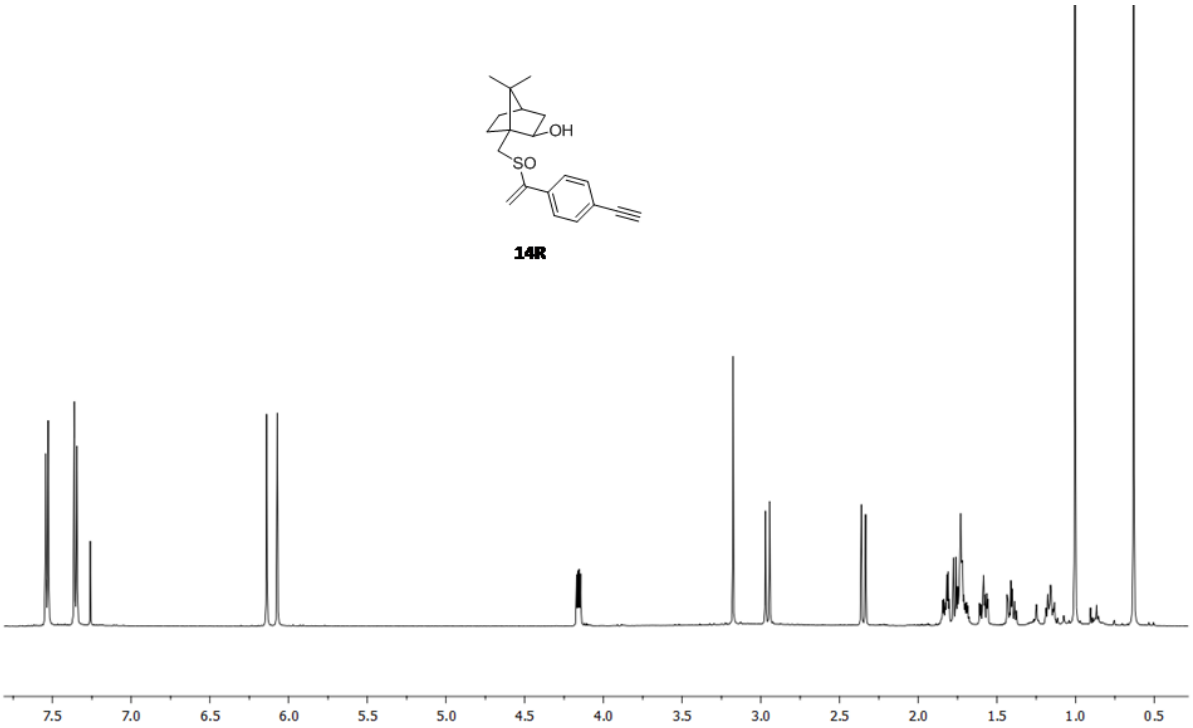

OH
S
CO2Me
12
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20

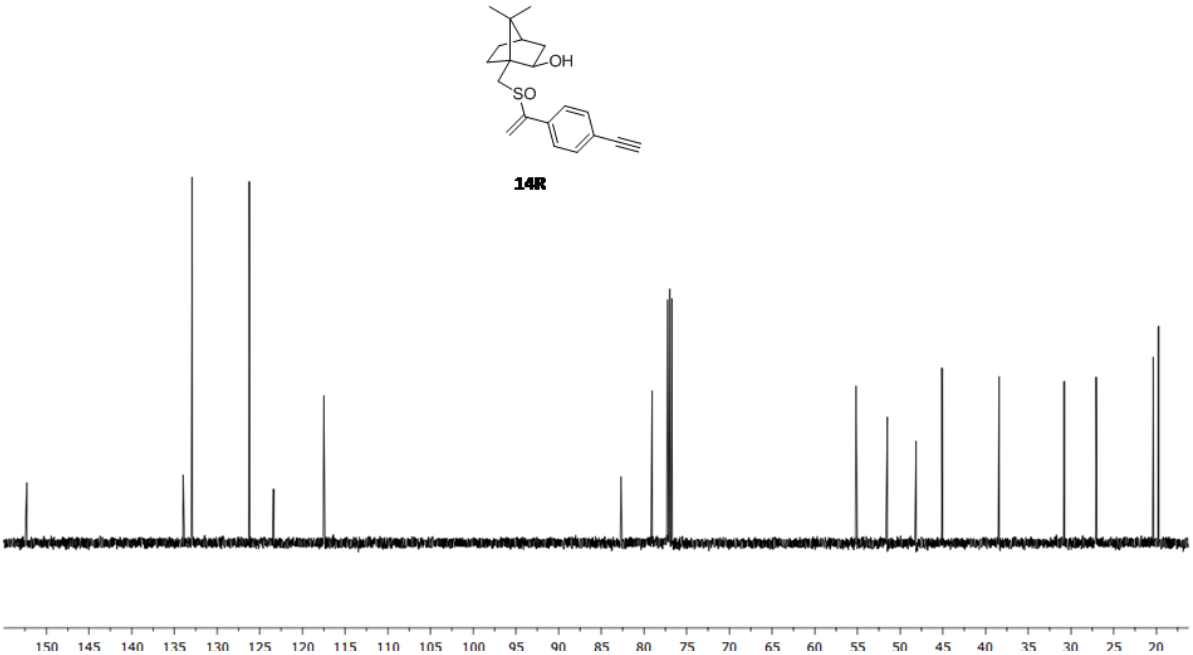

OH
S
O
CO2Me
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8

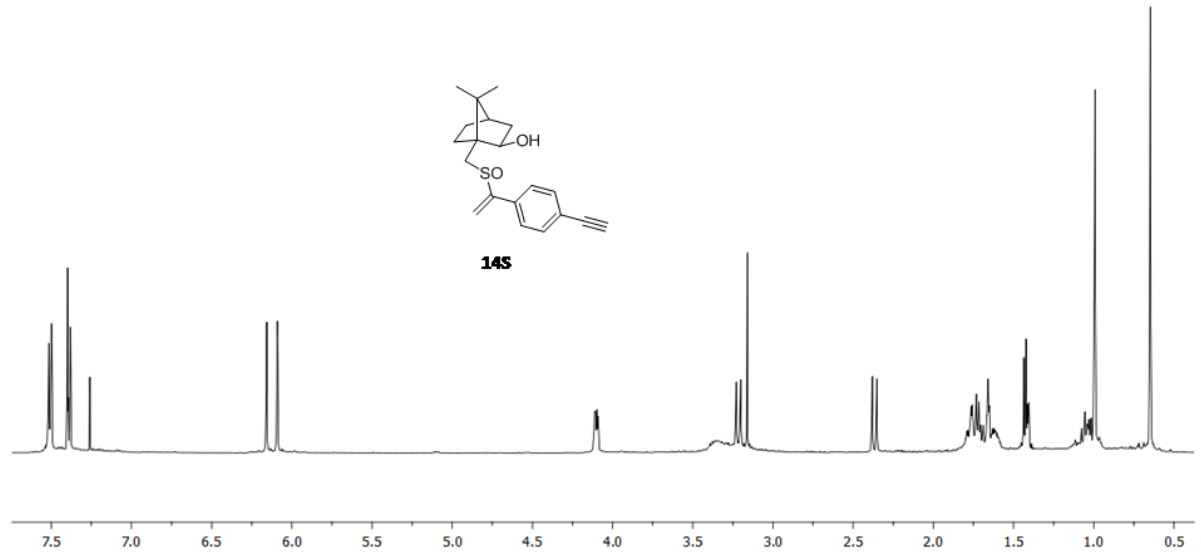
13


OH
S
O
CO2Me
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

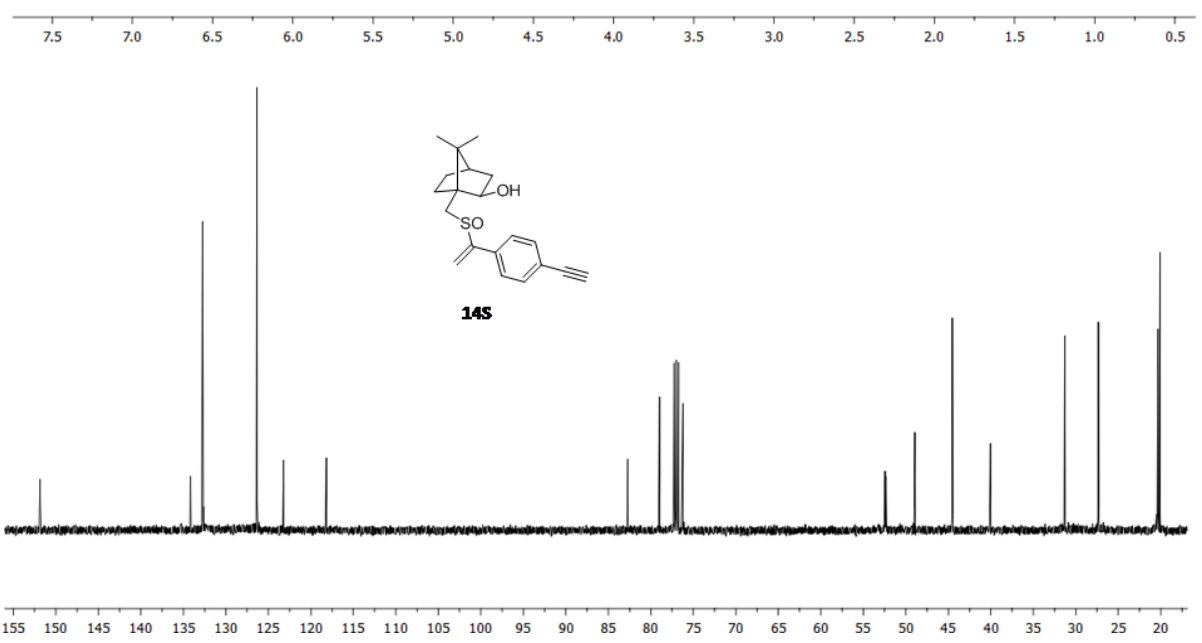
13


OH
SO
14R
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

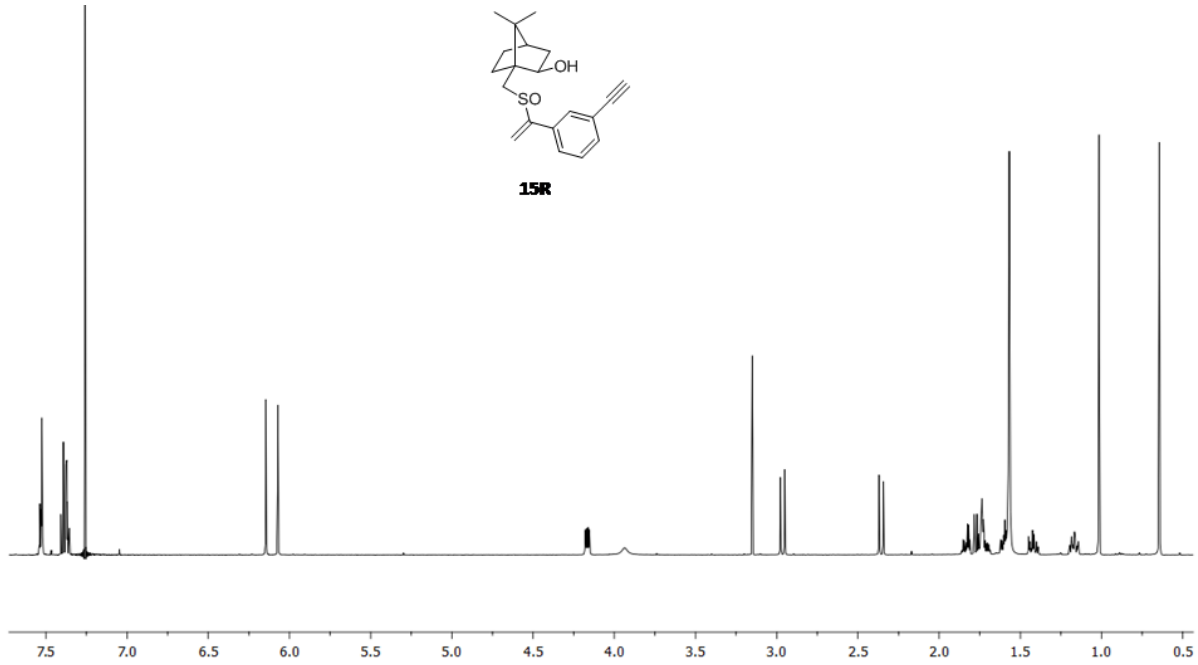

OH
SO
14R
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20



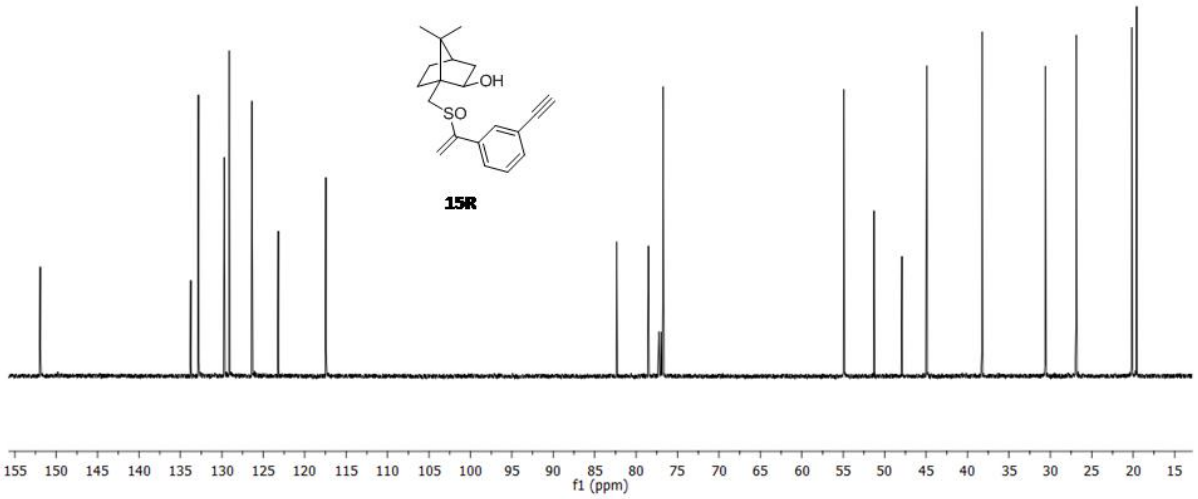
14S



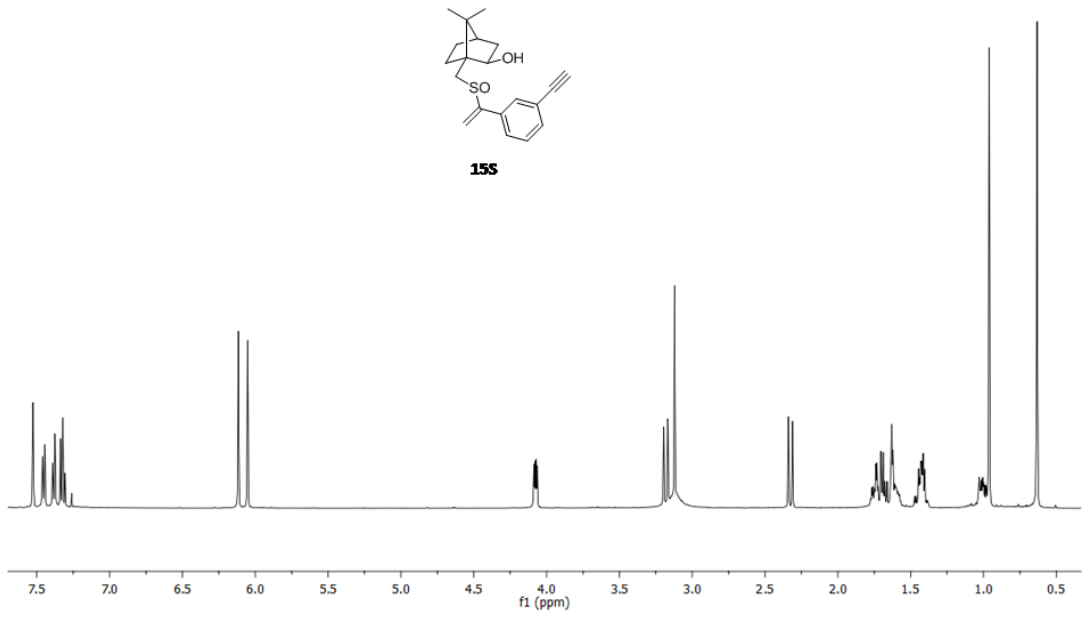
14S


OH
SO
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

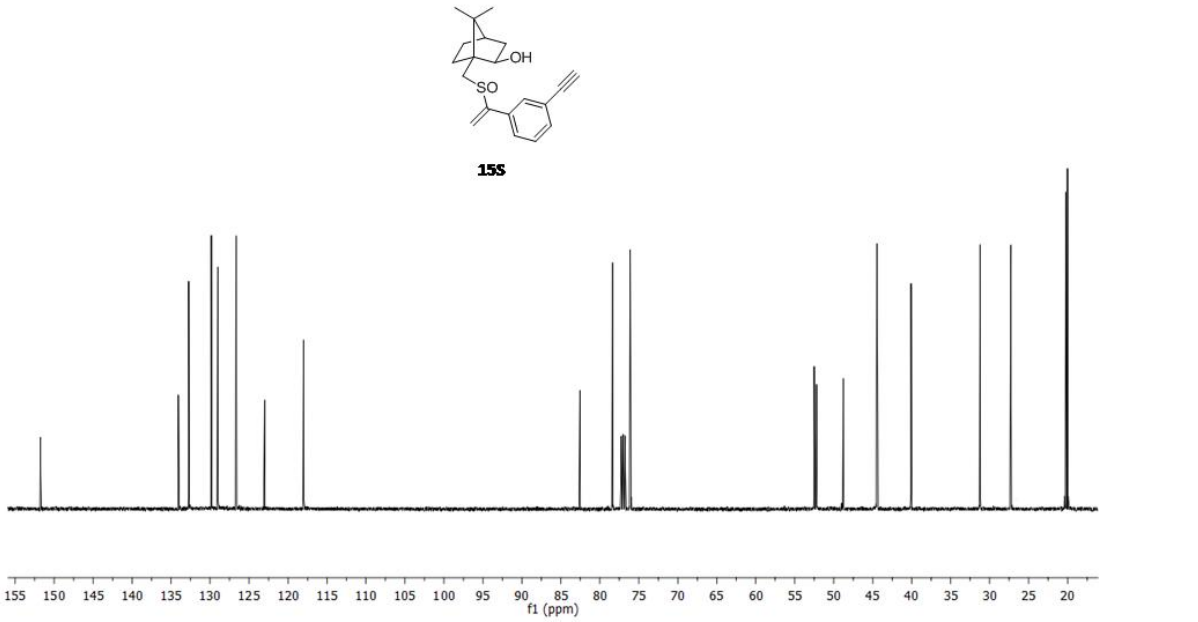
15R

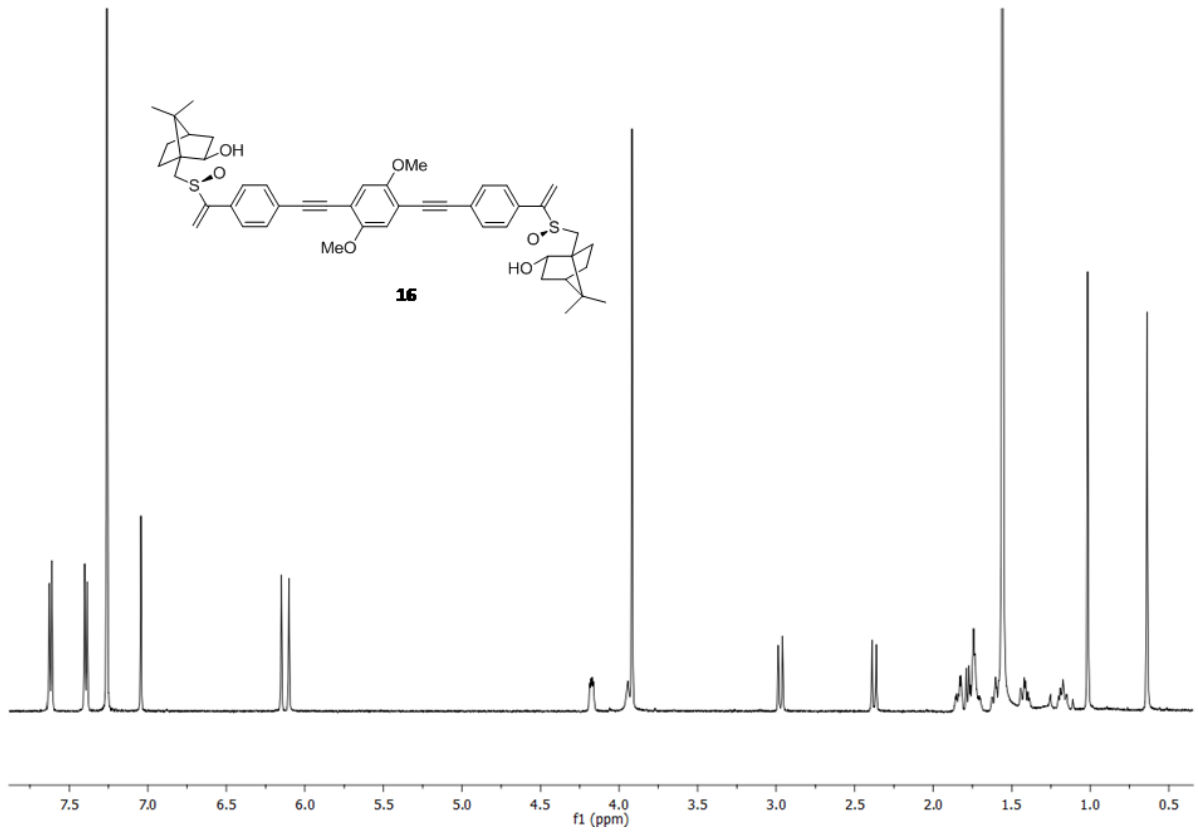

OH
SO
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
f1 (ppm)

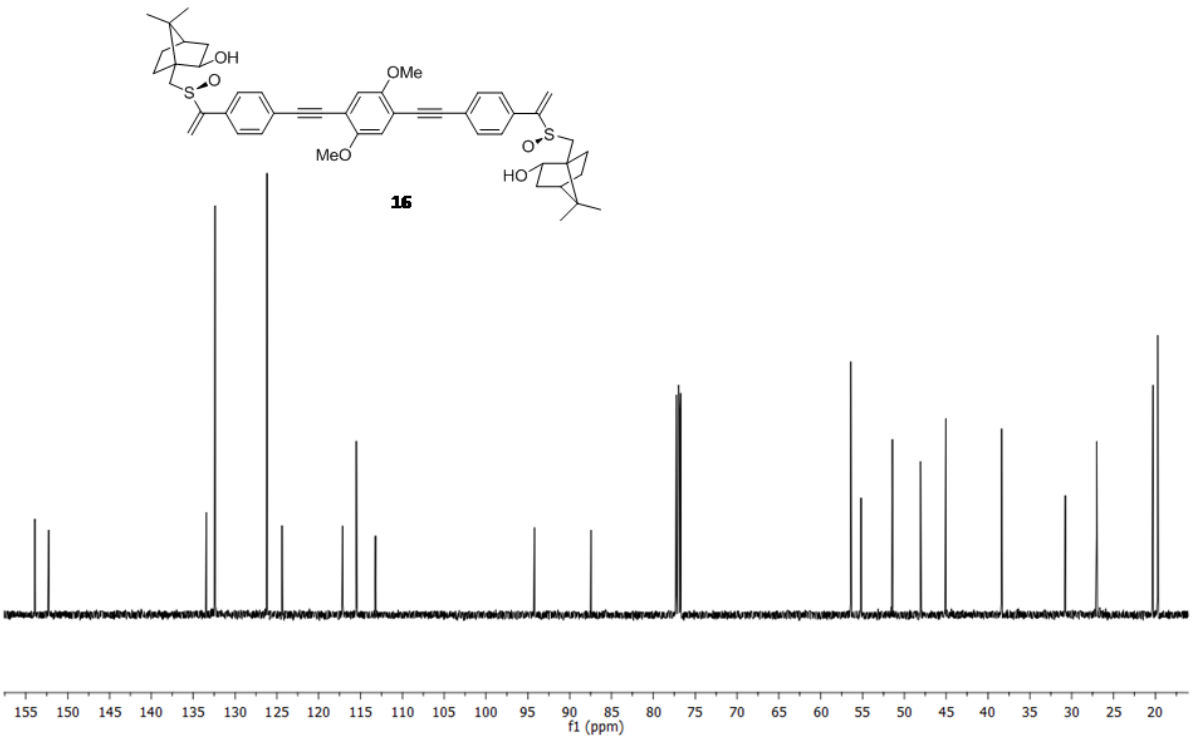
15R

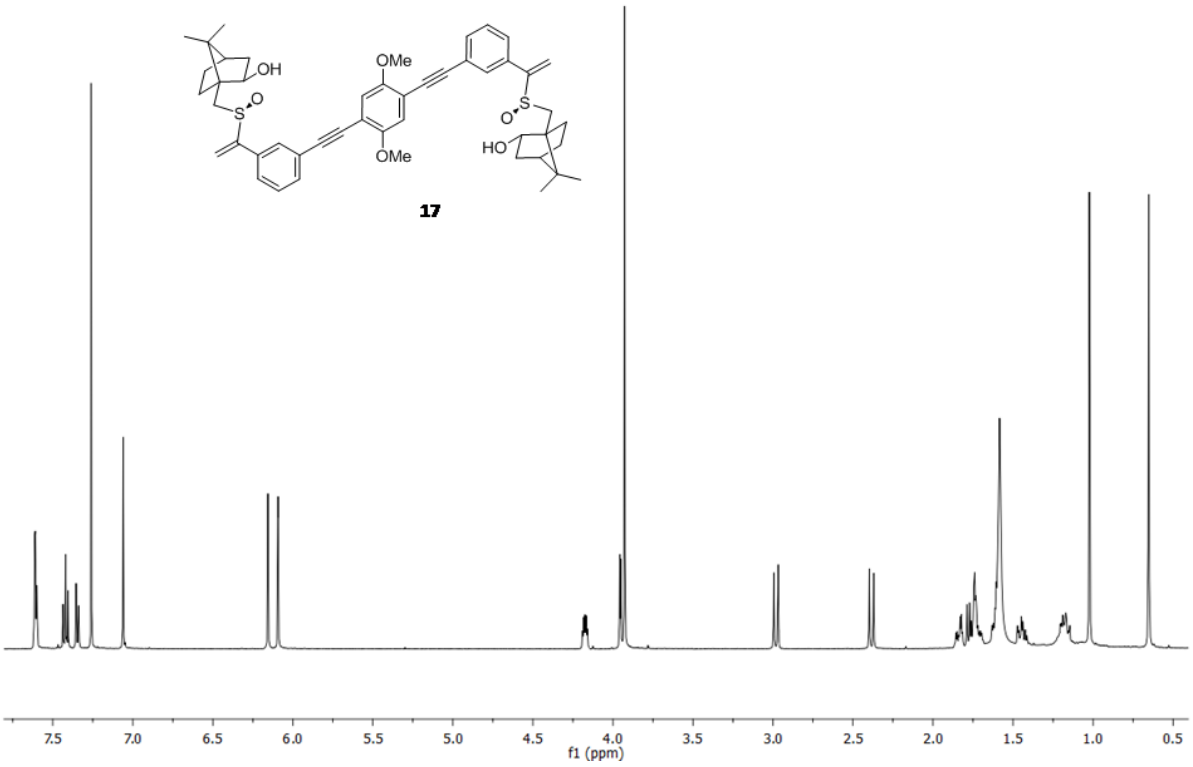

OH
SO
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

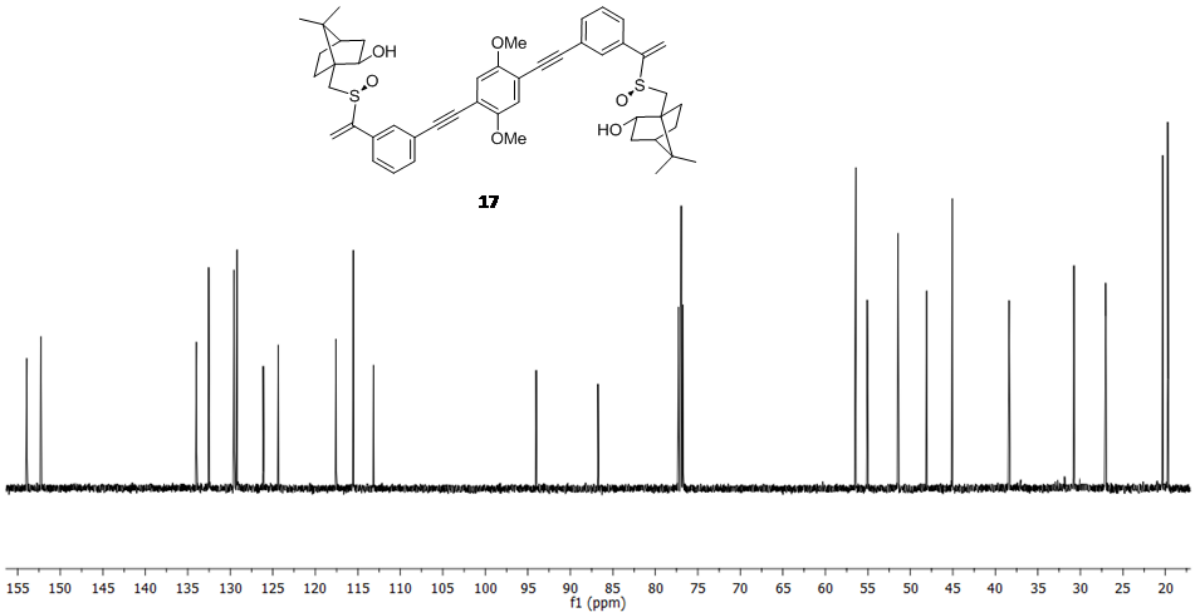
15S


OH
SO
155
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)

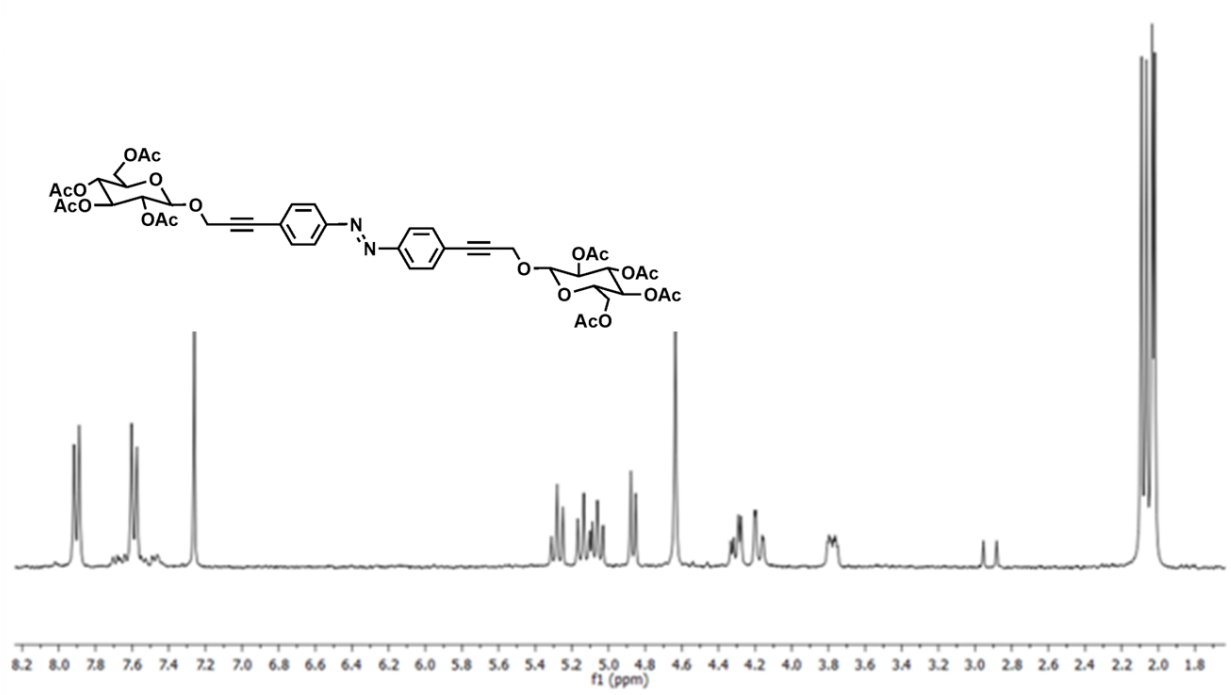

OH
S
O
OMe
MeO
O
S
HO
16
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

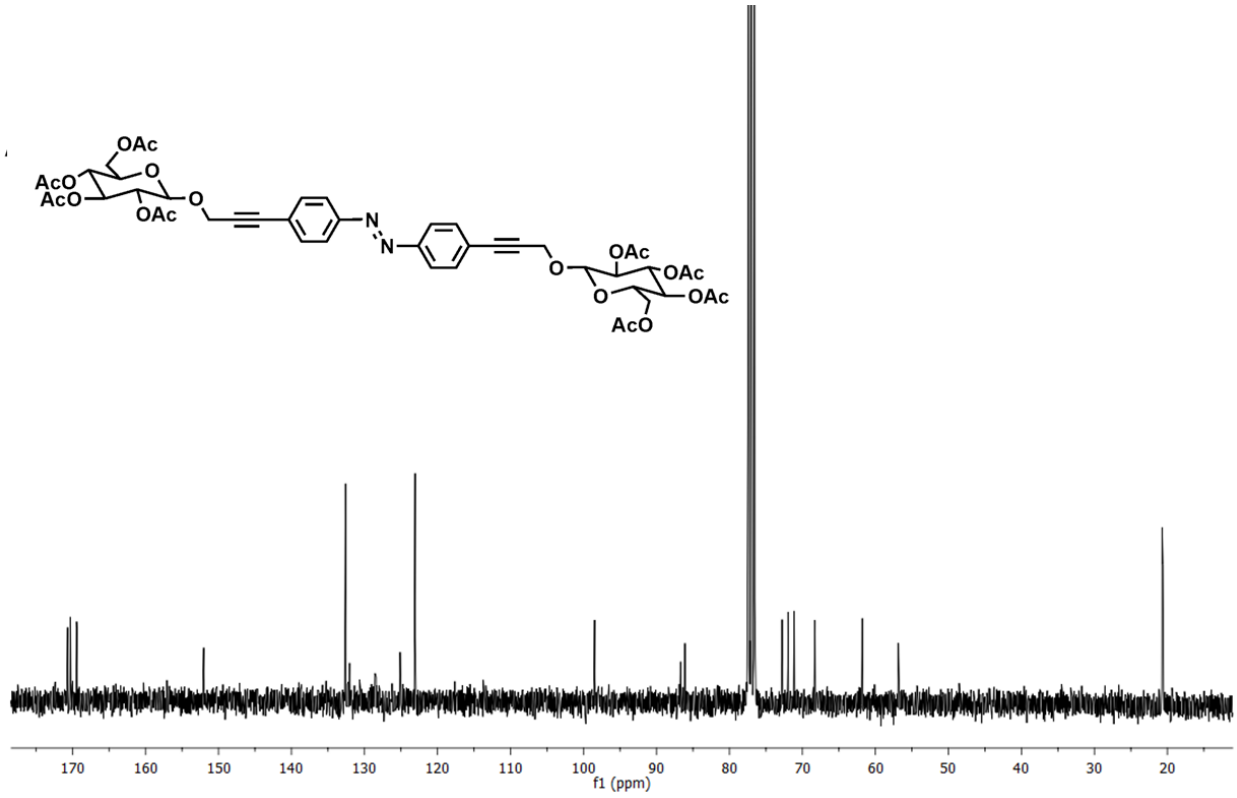

OH
S
O
OMe
MeO
O
S
HO
16
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)

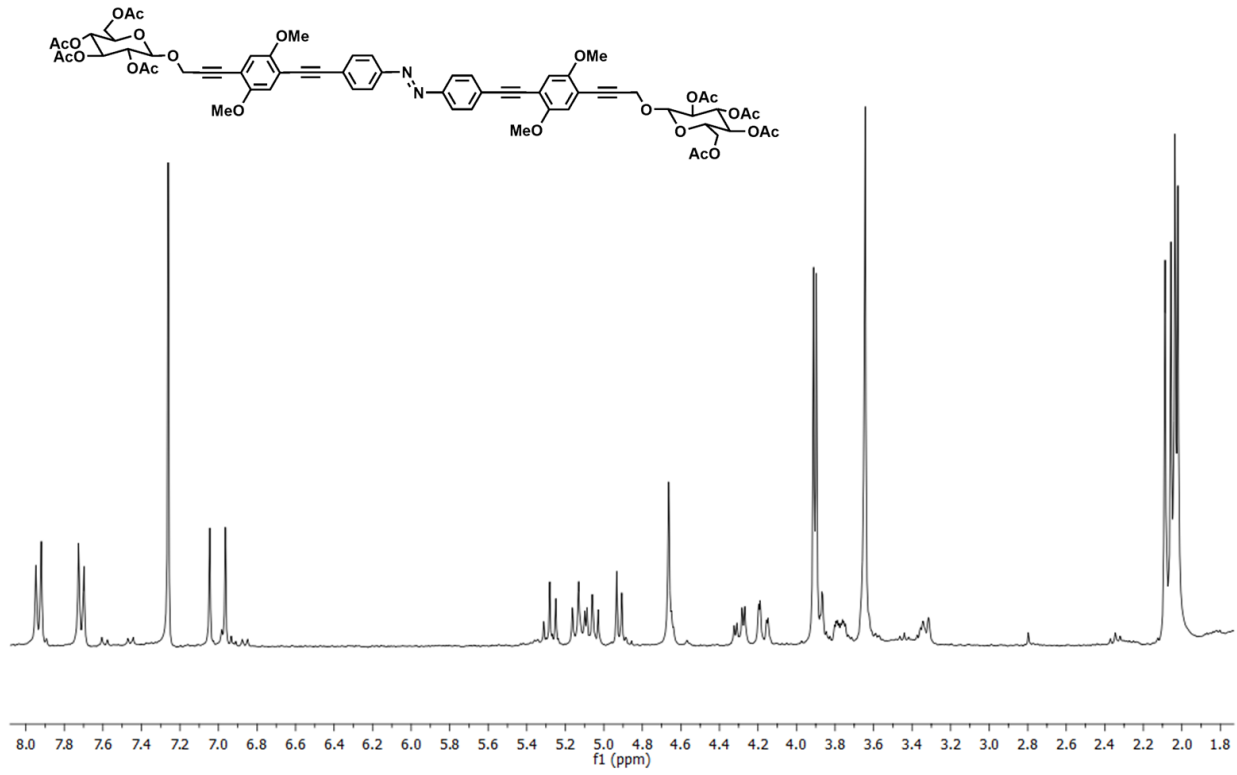

OH
S
O
OMe
OMe
O
S
HO
17
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

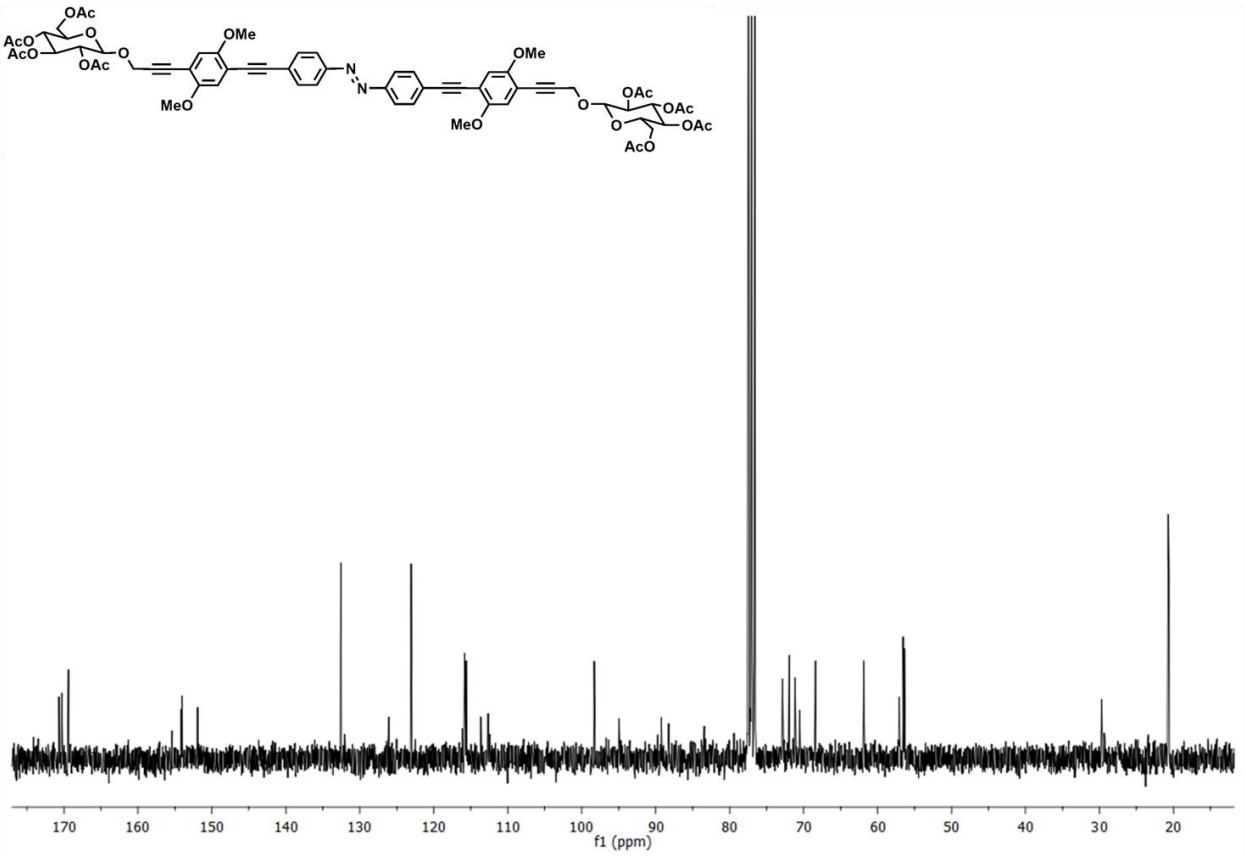

OH
OMe
S
O
OMe
O
S
HO
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)

17


OAc
AcO
AcO
OAc
O
N
N
O
OAc
OAc
OAc
O
AcO
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
f1 (ppm)


OAc
AcO
AcO
OAc
O
O
N
N
OAc
OAc
OAc
O
O
AcO
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)


OAc
AcO
AcO
OAc
O
OMe
MeO
N
N
OMe
MeO
O
OAc
OAc
OAc
O
AcO
8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
f1 (ppm)


OAc
AcO
AcO
OAc
O
OMe
MeO
N
N
OMe
MeO
O
OAc
OAc
OAc
O
AcO
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)